

Azithromycin to Prevent Bronchopulmonary Dysplasia in *Ureaplasma*-infected Preterms

A Phase IIb randomized, placebo-controlled, double-blind trial of azithromycin to eradicate *Ureaplasma* respiratory tract infection in preterm infants

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Statement of Compliance

This study will be carried out in accordance with the US Code of Federal Regulations (CFR), local regulations, and Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46; and 21 CFR including part 50 and 56 concerning informed consent and IRB regulations, and 21 CFR 11 concerning electronic records.
- International Conference on Harmonisation (ICH E6); 62 Federal Register 25691 (1997)
- NICHD Clinical Terms of Award

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any subjects. CVs for all investigators and sub-investigators participating in this trial are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).

- **Signature Page 1**

The signature below constitutes approval of this protocol and the attachments, and provides the required assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable US federal regulations and (ICH E6) guidelines.

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The Lead Principal Investigator (Protocol Chair) should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of Regulatory documents and a copy should be maintained at the site.

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The Principal Investigator or Investigator(s) of Record from each participating clinical site should sign the Signature Page 2 as appropriate. This Signature Page 2 should be maintained at each clinical site.

Tool Revision History

Version Number 1.2

Version Date 1/10/13

Summary of Revisions Made:

Version Number 1.3

Version Date 3/8/13

Summary of Revisions Made (Changes in italics):

1. Added List of Case Report Forms to be used as source documents (section 10.2)
2. Updated Consent Form to include time of consent to investigator signature line
3. Provided updated Case Report Forms in Appendix F

Version Number 1.4

Version Date 3/21/2013

Summary of Revisions Made:

1. Changed the timing of long-term follow-up from 18-22 months to 22-26 months adjusted age
2. In response to FDA Drug Safety Communication dated 3/12/2013 concerning Azithromycin and the risk of potentially fatal heart rhythms, have added conditions that may increase the risk for prolonged QT in stopping rules for individual subjects (summarized in Background p. 14-15 and Section 5.1.4).
3. Consent form procedures section revised to include the conditions that may increase the risk for prolonged QT that would cause delay in drug receipt or discontinuation of the drug.
4. Corrected the timing of the hearing screen and physiologic BPD assessment to 36 weeks or pre-discharge in study schedule (Table 3).
5. Revised consent process, to include parental consent for banking (storage) of *Ureaplasma* samples isolated from positive subjects and contact for future studies (sections 4.3 and 6.2.2).

Version Number 2.0

Version Date 4/14/2014

Summary of Revisions Made:

1. Change in eligibility criteria: Eliminated appropriate for gestational age (AGA) from inclusion criteria. Ten percent of potential subjects <29 weeks gestation are small for gestational age (SGA). Based on 2 prior prospective studies of *Ureaplasma* respiratory colonization, the risk of *Ureaplasma* respiratory colonization and BPD are similar for AGA and SGA infants <29 weeks when delivery for maternal indications are excluded. This should not impact risks for subjects and should not significantly change the anticipated respiratory colonization rate of enrolled subjects.
2. Added additional pulmonary follow-up at 12 months adjusted age to section 6.3.5, study schedule and consent form procedures section.
3. Clarify calculation of dosing volumes for study drug (Section 5.2.4)
4. Revised serious adverse event reporting procedures (Section 7.4) for all SAEs regardless of relationship to study drug to be reported to the sponsor within 5 business days. The sponsor will forward all SAE reports to DSMB chair who will review and

determine whether to request treatment assignment. A monthly cumulative summary of SAEs will be submitted to the DSMB chair as previously described.

5. Specific requirements for safety reports for NEC cases are included in section 7.4.
6. Clarify that subjects will be followed for safety (morbidity of prematurity, adverse events, serious adverse events) until 44 weeks post-menstrual age (PMA), discharge, or transfer whichever comes first.
7. Added Appendix E. Severity Assessment of Clinical Laboratory Values
8. Oregon Health and Science University has been dropped as a participating site.

Version Number 3.0

Version Date 5/28/2015

Summary of Revisions Made:

1. Added information to risk section (p. 18 and 38) concerning new published information concerning an association of oral azithromycin and increased risk for idiopathic hypertrophic pyloric stenosis in infants. The consent form was revised to include this new information (see page 65).
2. Since additional planned azithromycin drug measurements have been completed, residual blood collection procedure will be discontinued.
3. Added Christiana Care Health System, Newark, DE as a study site.

Version Number 4.0

Version Date 1/13/2016

Summary of Revisions Made:

1. Gestational age eligibility criteria change: This revision changes the gestational age (GA) eligibility from 24⁰-28⁶ wk to 24⁰-26⁶ wk. This change is requested with approval from the DSMB to limit enrollment to the gestational age stratum with the highest *Ureaplasma* respiratory colonization rate and to reduce the exposure of non-colonized infants to the potential risks associated with azithromycin. To date, 94 infants have been enrolled in the trial; 60 subjects were in the lower gestational age stratum 24⁰-26⁶ wk and 34 subjects were in the higher gestational age stratum 27⁰-28⁶ wk. The *Ureaplasma* respiratory colonization rate at study entry was 54% in the lower GA stratum in contrast to 16% colonization rate in more mature infants in the higher GA stratum. Using a 2-sided α level of 0.05 and assuming 20% of subjects are twins and 5% drop-out rate, the study will have power of 0.8 to detect a 40% difference in the primary outcome of survival with *Ureaplasma* clearance with enrollment of 30 *Ureaplasma*-positive infants in each group (See Table 6, Section 9.2, p. 46). The change in eligibility criteria to limit enrollment to the lower GA stratum will reduce the number of total enrollment from the originally planned 180 to 140 to obtain 60 *Ureaplasma*-positive subjects. All infants enrolled and randomized in the trial including those already enrolled with gestational age 27⁰-28⁶ will be followed until 22-26 month adjusted age. Revisions have been underlined in the text.

Version Number 5.0

Version Date 1/30/2017

Summary of Revisions Made:

1. Added Ages and Stage Questionnaire-3 (ASQ-3) as Neurodevelopmental Assessment Tool: Since some subjects are unable to return for in-person neurodevelopmental assessments (neuromotor exam and BSID-III), the ASQ-3 parental questionnaire has

been added as an option since it can be administered by phone, is easily scored, and has high sensitivity and negative predictive value to detect neurodevelopmental impairment in preterm infants <32 weeks gestation at 22-26 months adjusted age ¹ (page 39).

2. Adjusted windows for follow-up assessment: Due to the challenges in reaching some parents within the windows for pulmonary assessments at 6, 12, and 22-26 months adjusted age, and neurodevelopmental assessments at 22-26 months adjusted age, the window for the questionnaires now extend from 5 months adjusted age for the 6 month pulmonary assessment through 30 months adjusted age (page 38).
3. Cranial Ultrasound (CUS) and Retinopathy of Prematurity (ROP) results confirmation: In order to confirm accuracy and completeness of CUS and ROP results, all CUS and ROP reports for all subjects will be reviewed blinded to treatment assignment during site visits. In addition, independent review of CUS images may be requested for confirmation if there are differences in rates of severe IVH (>Grade 2) by site or treatment assignment (page 41).

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PRÉCIS

Study Title: A Phase IIb randomized, placebo-controlled, double-blind trial of azithromycin to eradicate *Ureaplasma* respiratory tract infection in preterm infants

Objectives

Ureaplasma spp. respiratory tract colonization is a risk factor for the development of the chronic lung disorder bronchopulmonary dysplasia (BPD) in preterm infants ^{2,3}.

Preliminary data suggests that *Ureaplasma*-colonized infants may be at continued increased risk for adverse pulmonary outcomes in the first year of life. Trials of antibiotic therapy with erythromycin in the first few weeks of life often failed to eradicate respiratory colonization and the development of BPD in *Ureaplasma*-infected infants ⁴⁻⁶.

There are no current effective therapies for the treatment of *Ureaplasma* in preterm infants. Our long-term objective is to develop therapies to eradicate *Ureaplasma* from the lungs of preterm infants and prevent or ameliorate *Ureaplasma*-mediated lung inflammation, injury, altered developmental signaling, and fibrosis.

The azalide antibiotic azithromycin (AZI) has immunomodulatory properties that make it an ideal candidate for therapy to prevent *Ureaplasma*-mediated lung injury in preterm infants. Azithromycin 1) inhibits neutrophil influx and chemoattractant/cytokine release in murine lung injury models ⁷⁻⁹; 2) exhibits higher potency than erythromycin against clinical *Ureaplasma* isolates *in vitro* ¹⁰; and 3) is preferentially concentrated in alveolar epithelial lining fluid and macrophages ^{11,12}. In a neonatal murine *Ureaplasma* infection model, AZI, but not erythromycin treatment, improved survival and reduced lung inflammation ¹³.

We hypothesize that intravenous (IV) AZI therapy will prevent BPD in *Ureaplasma*-colonized preterm infants by accelerating pathogen clearance and/or down-regulating the pulmonary inflammatory response. We conducted a Phase I open-label, pharmacokinetic (PK) study characterizing the single dose PK, safety, tolerability, and biologic effects of 10 and 20 mg/kg IV AZI in mechanically ventilated 24-28 wk gestation preterm neonates who are at high-risk for *Ureaplasma* respiratory tract colonization and BPD. Pharmacokinetic data from 25 neonates (12 dosed with 10 mg/kg iv and 13 dosed with 20 mg/kg iv) were analyzed using a population modeling approach. A two-compartment structural model with the clearance and volume of peripheral compartment (V₂) allometrically scaled on body weight best described the PK of AZI in preterm neonates. Although both single dose regimens appeared to be safe, the single 10 mg/kg dose was insufficient to eradicate *Ureaplasma* or to suppress pulmonary inflammatory responses. Pharmacokinetic simulations indicate that even multiple dose administration of 10 mg/kg AZI would be inadequate to maintain AZI plasma concentrations above the *Ureaplasma* MIC₅₀. Despite apparent clearance of ureaplasmas in all infected subjects treated with 20 mg/kg single dose compared to 43% failure rate in the 10 mg/kg group ¹⁴, simulations suggest that multiple azithromycin dose regimens may be needed to maintain a plasma concentration of azithromycin above MIC₅₀ to clear the organisms.

We then enrolled 15 subjects from 6 sites in a Phase IIa open-label, pharmacokinetic study characterizing the PK, safety, and biologic effects of 20 mg/kg x 3 days AZI regimen. Although there was no difference in physiologic BPD rates among the three azithromycin dose groups, the duration of supplemental oxygen was significantly shorter and there was a trend towards shorter duration of mechanical ventilation for the 20

mg/kg multi-dose group. Compared to the single dose groups, the 20 mg/kg multi-dose effectively eradicated the *Ureaplasma* in all subjects who were colonized pre-dose. The 20 mg/kg x 3 day regimen appeared safe, with no deaths or serious adverse events attributed to the drug. The aims of the current protocol will address the next steps preparatory to Phase III safety and efficacy trials of AZI in the preterm population. The study population includes infants 24⁰-26⁶ weeks gestation due to the high rate of *Ureaplasma* spp. respiratory tract colonization and BPD in this subpopulation.

Aim 1: To determine the safety and microbiological efficacy of a multiple dose course of IV AZI to eradicate respiratory tract *Ureaplasma* infection that might lead to physiologic BPD in high risk preterm neonates in a Phase IIb multi-center, randomized, double-blind, placebo-controlled trial.

- **Primary outcome:** To compare the *Ureaplasma* clearance rate in AZI-treated vs placebo-treated infants
- **Secondary Outcomes:** To analyze the potential of multiple dose AZI to prevent physiologic BPD sufficiently to justify a Phase III clinical trial
 - To evaluate the relationship of microbiologic eradication of *Ureaplasma* respiratory tract colonization in AZI-treated infants and clinical outcome of physiologic BPD at 36 wks post-menstrual age
 - To analyze the effect of IV AZI on other secondary endpoints including overall mortality, number of days of mechanical ventilation and oxygen supplementation, exposure to non-study antibiotics, and incidence of co-morbidities of prematurity

Aim 2: To refine the population PK model of the multiple doses of azithromycin in preterm infants 24-28 weeks gestation.

Aim 3: To analyze the potential of multiple dose AZI to improve the pulmonary outcomes at 6, 12, and 22-26 months adjusted age assessed as number and duration of hospitalizations, number of physician and emergency visits for respiratory illness, frequency of pulmonary medication use, and Infant Lung Health Questionnaire parental report of recurrent wheezing or chronic cough.

Aim 4: To assess the impact of multiple dose AZI on risk for neurodevelopmental impairment at 22-26 months adjusted age.

Design and Outcomes

The study design will be a double-blind, placebo-controlled clinical trial to test the efficacy and safety of azithromycin 20 mg/kg x 3 days to eradicate *Ureaplasma* spp from the respiratory tract of preterm infants 24⁰-26⁶ weeks gestation exposed to positive pressure ventilation. The primary outcome will be survival with microbiological eradication of *Ureaplasma* defined as survival to discharge or transfer with 3 negative cultures obtained post-therapy. Secondary outcomes will include physiologic BPD at 36 weeks post-menstrual age (PMA), overall mortality, incidence of co-morbidities of prematurity such as intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, bacterial and fungal nosocomial infection, pulmonary air leak, patent ductus arteriosus, retinopathy of prematurity, number of days of positive pressure ventilation, number of days of oxygen supplementation, use of postnatal steroids, and use of non-study antibiotics.

Interventions and Duration

After obtaining parental consent, 2 tracheal aspirates and one nasopharyngeal aspirate will be obtained at least 2 hours apart for *Ureaplasma* culture and PCR and the infant randomized to either placebo or azithromycin 20 mg/kg IV x 3 days. Follow-up cultures will be obtained 2 and 4-5 days post last dose and at 21d postnatal age (PNA). Additional tracheal aspirates for inflammatory cytokine measurements will be obtained on the same schedule if the infant remains intubated. We have completed collection of 1-3 samples of residual blood specimens between 4-6 h, 8-24 h, and 69-120h post first-dose from a sub-group of 30 subjects. Subjects will be followed to discharge for safety. Physiologic BPD will be assessed at 36±1 wk PMA by a timed-oxygen reduction test. At 6, 12, and 22-26 months adjusted age, a pulmonary outcome questionnaire will be administered by phone or in person interview. At 22-26 months adjusted age, neurodevelopmental outcomes will be assessed by 1) Bayley Scale of Infant and Toddler Development, 3rd edition (BSID-III); 2) Amiel-Tison neurologic examination; 3) Gross Motor Function Classification System; and 4) medical record review for hearing impairment with or without amplification and vision impairment (vision <20/200). *For infants who are unable to return for an inpatient assessment, the Ages and Stages Questionnaire-3 (ASQ-3) will be administered by phone.*

Sample Size and Population

We will limit the recruitment after January 2016 to infants 24⁰-26⁶ wk gestation who are at highest risk for *Ureaplasma* respiratory tract colonization and BPD. All admissions to the NICUs of the 7 clinical sites <72 h age with GA 24⁰-26⁶ wk will be screened for study eligibility and parental consent of eligible subjects will be obtained. With a 54% colonization rate, a total of 140 subjects will be enrolled to achieve 30 *Ureaplasma*-positive subjects in each arm. Subjects will be stratified by center. Infants will be randomized according to a permuted-block design to receive azithromycin or placebo.

1. STUDY OBJECTIVES

1.1 Primary Objective

To determine the safety and microbiological efficacy of a multiple dose course of IV azithromycin to eradicate respiratory tract *Ureaplasma* infection that might lead to physiologic BPD in preterm neonates in a Phase IIb multicenter, randomized, double-blind, placebo-controlled clinical trial. We hypothesize that intravenous (IV) AZI therapy will prevent BPD in *Ureaplasma*-colonized preterm infants by accelerating pathogen clearance and/or down-regulating the pulmonary inflammatory response.

1.2 Secondary Objectives

To analyze the potential of early multiple dose azithromycin to prevent physiologic defined BPD

- To evaluate the relationship of microbiologic eradication of *Ureaplasma* respiratory tract colonization in azithromycin treated infants and clinical outcome of physiologic BPD at 36 wks post-menstrual age
- To analyze the effect of IV azithromycin on other secondary endpoints including overall mortality, number of days of mechanical ventilation and oxygen supplementation, exposure to non-study antibiotics, and incidence of co-morbidities of prematurity

To refine the population PK model of the multiple doses of azithromycin in preterm infants 24-28 weeks gestation.

To analyze the potential of multiple dose AZI to improve the pulmonary outcomes at 6, 12, and 22-26 months adjusted age assessed as number and duration of hospitalizations, number of physician and emergency visits for respiratory illness, frequency of pulmonary medication use, and Infant Lung Health Questionnaire parental report of recurrent wheezing or chronic cough.

To analyze the impact of multiple dose AZI on risk for neurodevelopmental impairment at 22-26 months adjusted age.

2. BACKGROUND AND RATIONALE

2.1 BPD: Major Morbidity of Prematurity

Bronchopulmonary dysplasia (BPD) was first described over 40 years ago as a progression of characteristic chest radiographic findings that correlated with pathologic changes of acute and chronic lung inflammation, fibrosis and bronchial smooth muscle hypertrophy in premature, ventilator-dependent infants^{15,16}. With recent improvements in perinatal care, BPD has become a disease limited to the most immature infants¹⁷, occurring in 30% of infants ≤ 28 wks gestation, but in only 3% of infants >28 weeks¹⁸. Compared to the lung histology observed in the ventilated preterm lung during the pre-exogenous surfactant era, the 'new' BPD is characterized by more uniform inflation, fewer but larger alveoli, and less fulminant, but persistent inflammation¹⁹. Studies of human infants and experimental animal models indicate that the central event in BPD pathogenesis is the interruption of normal developmental signaling during early stages of lung development by lung injury that is often initiated in utero by intrauterine infection and a subsequent dysregulated inflammatory response¹⁷.

Despite considerable effort focused on reducing BPD in infants ≤ 28 wks gestation who are at highest risk, recently studied therapies (post-natal steroids²⁰, vitamin A²¹, caffeine^{22,23}, inhaled nitric oxide^{24,25}, and early CPAP^{26,27}) have had only modest impacts on the incidence and severity of BPD. Furthermore, the reduction in early pulmonary morbidity may come at the cost of higher neurodevelopmental handicaps later in life²⁸. The effects of these therapies on later pulmonary health are currently being evaluated^{29,30}. None of these therapies address the potential contribution of infections to BPD.

Persistent *Ureaplasma* respiratory tract colonization: Risk factor for BPD:

Ureaplasma parvum (serotypes 1, 3, 6, and 14) and *U. urealyticum* (serotypes 2, 4, 5, 7-13) are closely related species of the *Mollicutes* class that are among the smallest free-living, self-replicating cells^{2,31,32}. All serovars lack cell walls, exhibit limited biosynthetic abilities, hydrolyze urea to generate ATP, and adhere to human mucosal surfaces². *U. parvum* is the most common species isolated from clinical specimens^{2,33}. Although *Ureaplasma* spp. are considered commensals in the adult GU tract, they have been implicated in complications of human pregnancy and neonatal outcomes. *Ureaplasma* urogenital tract colonization has been causally linked to infertility^{34,35}, early pregnancy loss³⁶, stillbirth^{37,38}, and preterm birth^{39,40}. *Ureaplasma* spp. are the most common organisms isolated from amniotic fluid obtained from women who present with preterm labor (POL) with intact membranes⁴¹, preterm premature rupture of

membranes⁴², and short cervix associated with microbial invasion of the amniotic cavity⁴³, and from infected placentas⁴². Recovery of *Ureaplasma* from the chorion increased with duration of rupture membranes, suggesting an ascending route of infection³⁹. However, *Ureaplasma* has also been detected in 22% of placentas with duration of membrane rupture < 1h, suggesting the possibility of a pre-existing infection. Indeed, *Ureaplasma* species have been detected in amniotic fluid as early as the time of genetic amniocentesis (16-20 weeks) in up to 13% asymptomatic women⁴⁴⁻⁴⁷. Placentas with the lowest rate of *Ureaplasma* recovery were from women delivered for pre-eclampsia or intrauterine growth restriction³⁹.

Vertical transmission is the only known route of infection in the newborn. The rate of respiratory tract colonization with *Ureaplasma* in infants <1500g birth weight ranges from 20-45%, depending on study entry criteria, frequency of sampling and detection methods^{48,49}. In a recent cohort of infants <33 wks gestation, *Ureaplasma* spp. were detected during the first week of life in tracheal aspirates or nasopharyngeal specimens in 35% of infants⁵⁰. *Ureaplasma* respiratory tract colonization is associated with a peripheral blood leukocytosis⁵¹ and early radiographic emphysematous changes of bronchopulmonary dysplasia (BPD)^{52,53}. These findings may be explained, in part, by an *in utero* onset of the inflammatory response and lung injury. The contribution of *Ureaplasma* respiratory tract colonization to the development of BPD has been debated; however, a meta-analysis of 17 clinical studies published before 1995 supported a significant association between *Ureaplasma* respiratory tract colonization and development of BPD defined as oxygen dependence at 28 to 30 d postnatal age⁴⁸. In a meta-analysis of 36 published studies involving ~3000 preterm infants, Schelonka *et al.*⁴⁹ observed a significant association between *Ureaplasma* respiratory colonization and development of BPD whether defined as oxygen-dependence at 28 d or at 36 wk post menstrual age (PMA). Studies published since the last meta-analysis support the association between *Ureaplasma* respiratory colonization and BPD^{54,55}, particularly for the subset of *Ureaplasma*-colonized infants exposed to chorioamnionitis and leukocytosis at birth⁵⁵. Some *Ureaplasma* serovars have been implicated in adverse pregnancy outcomes more commonly than others⁵⁶⁻⁵⁹. Although *U. parvum* is more commonly isolated from clinical specimens^{57,59}, there is a higher rate of BPD in *U. urealyticum* colonized infants⁵⁷. **Whether there are differences in the *in vivo* response to antibiotic therapy among the serovars or species of *Ureaplasma* is unknown.**

***U. urealyticum* modulates the pulmonary immune response:** Evidence from studies of human preterm infants⁶⁰, and intrauterine infection models in mice⁶¹, sheep^{62,63}, and non-human primates^{64,65} as well as *in vitro* studies⁶⁶⁻⁶⁹ support that *Ureaplasma* infection is pro-inflammatory and pro-fibrotic and results in a BPD phenotype. In a review of lung pathology of archived autopsy specimens from *Ureaplasma*-infected preterm infants, we observed moderate to severe fibrosis and increased elastin fiber accumulation in all *Ureaplasma*-infected infants compared to non-infected gestational controls and infants who died with pneumonia from other causes^{70,71}. There were increased numbers of myofibroblasts and TNF-alpha and transforming growth factor β_1 (TGF β_1)-immunostaining in the lungs of all *Ureaplasma*-infected infants^{70,71}. The increase in fibrosis and elastin fiber accumulation in the distal lung correlated spatially and temporally with the presence of macrophages positive for TGF β_1 , suggesting that these are closely linked⁷¹. Preterm infants with *Ureaplasma* respiratory colonization have elevated tracheal aspirate IL-1 β , TNF-alpha, and monocyte chemoattractant protein-1 (MCP-1) concentrations and neutrophil chemotactic activity during the first

weeks of life compared to non-colonized infants^{60,72,73}. Experimental murine intrauterine *U. parvum* exposure stimulated fetal lung cytokine expression and augmented hyperoxia-induced lung injury⁶¹. In the 125 d immature baboon model, we observed sustained recruitment of neutrophils and macrophages into the lung, increased expression of pro-inflammatory (TNF-alpha, interleukin (IL)-1 β) and pro-fibrotic cytokines (TGF β ₁; oncostatin M), extensive fibrosis, and increased myofibroblast phenotype in lungs of antenatal *Ureaplasma*-infected animals compared to gestational controls or non-infected ventilated animals^{64,74}. In a study of rhesus macaques, histologic changes in the fetus' lungs depended on the duration of intrauterine exposure to *U. parvum*⁶⁵. Infection exposure duration less than 136 h resulted in neutrophil infiltration without epithelial injury. With progressive duration of exposure there was an influx of neutrophils and macrophages, epithelial necrosis, and type II cell proliferation. For exposure duration >10 d, increased collagen and thickened alveolar walls were evident. These data confirm that *Ureaplasma* infection contributes to chronic inflammation and fibrosis in the preterm lung. Moreover, these data suggest that *Ureaplasma* acts as a co-inflammatory stimulus by causing an augmented, dysregulated inflammatory response to subsequent inflammatory insults such as hyperoxia and volutrauma. **We propose that *Ureaplasma* may contribute to lung injury and fibrosis by modulating the local immune response to produce sustained chronic inflammation.**

Potential role of AZI to prevent *Ureaplasma*-mediated neonatal lung injury: Despite *in vitro* susceptibility of *Ureaplasma* to erythromycin⁷⁵ and favorable PK activity⁷⁶, trials of erythromycin therapy in *Ureaplasma*-colonized preterm infants have failed to demonstrate efficacy to prevent BPD^{4,5} or to eradicate respiratory tract colonization⁶. The failure to prevent BPD in these studies may have been due to the small sample size of each study, or to initiation of erythromycin therapy too late to prevent the lung inflammation and injury that contribute to the pathogenesis of BPD. Demonstration of efficacy of antibiotic therapy to prevent BPD in *Ureaplasma*-colonized preterm infants will require carefully designed, adequately powered studies.

The 14-membered macrolides that are derivatives of erythromycin and the related 15-member azalides have immunomodulatory effects, including effects on neutrophil function (e.g. chemotaxis, cell adhesion, oxidative burst, phagocytosis) and inhibition of release of cytokines involved in acute inflammation (e.g., IL-1 β , IL-8, TNF-alpha)⁷⁷⁻⁷⁹ and chronic inflammation (IL-12p40)⁸⁰ and NO₂⁻ production *in vitro*⁸¹. The macrolide antibiotics may exert these immunomodulatory effects in the setting of infection, and may occur independently of a direct bactericidal effect⁸². AZI inhibits neutrophil influx and chemoattractant/cytokine release in murine lung noninfectious^{7,9}, as well as pneumonia^{8,83} injury models. In addition, AZI exhibits higher potency than erythromycin against clinical *Ureaplasma* isolates *in vitro*^{10,75,84}. Pharmacokinetic studies in mice⁸⁵ and humans have shown that AZI is preferentially concentrated in pulmonary epithelial lining fluid and alveolar macrophages^{11,12}. Since neutrophil recruitment and activation has been implicated in BPD pathogenesis⁸⁶⁻⁸⁸, the experimental effects observed with AZI *in vitro* and *in vivo* indicate that this drug may be beneficial in the treatment of *Ureaplasma* infection and the prevention of BPD in preterm infants. **Taken together, these properties suggest that AZI is an ideal candidate for therapy to prevent *Ureaplasma*-mediated lung injury in preterm infants at high risk for BPD.** Since *Ureaplasma*-mediated lung injury may be initiated *in utero* and augmented postnatally by exposure to mechanical ventilation and hyperoxia, therapy to prevent BPD should be initiated as soon as possible after birth in infants at risk. This contention

was supported by the recent study by Walls *et al.*¹³ demonstrating that AZI, but not erythromycin prophylaxis improved outcomes and reduced inflammation in a murine neonatal *Ureaplasma* infection model. However, in a single center RCT pilot study, AZI (10 mg/kg/d x 7d followed by 5 mg/kg/d up to 6 wks) did not reduce the incidence of BPD at 36 wk PMA⁸⁹. In a follow-up larger trial, *Ureaplasma* clearance and BPD rates were similar in the placebo and AZI-treated groups⁹⁰. **These results underscore the importance of performing appropriate PK/pharmacodynamic studies preparatory to phase II/III trials to assess microbiological and clinical efficacy of AZI.**

Safety and Tolerance of AZI in the Pediatric Population: The safety and tolerability of oral AZI has been evaluated in 3995 patients aged 2 to 94⁹¹. The overall rate of side effects is 12% with gastrointestinal symptoms the most common. Ototoxicity, although rare, has been reported with IV AZI in adults and included reversible tinnitus and hearing loss⁹². A review of 43 open label, randomized pediatric trials performed outside the US that included 2655 patients treated with a 3 day course (10 mg/kg once daily) oral AZI for upper respiratory tract infections reported adverse effects in 8.7% subjects, primarily gastrointestinal symptoms⁹³. In a recent PK study of a single dose of IV AZI in 32 children ages 6 months to 16 years, there were no serious adverse events. Nausea was the most common treatment related symptom, reported in 25% patients.

Association of AZI with infantile hypertrophic pyloric stenosis: Although treatment with erythromycin in the first few months of life has been associated occasionally with the development of infantile hypertrophic pyloric stenosis (IHPS)^{93,94}, a recent report of an analysis of the association of prescriptions for oral azithromycin, erythromycin, or cephalexin during the first 90 days of life and diagnosis of pyloric stenosis (IHPS) in a retrospective cohort study of children born 2001-2012 utilizing the military health system database suggested an increased risk for IHPS with AZI exposure in the first 14 days of life⁹⁵. Of 1,074,236 infants reviewed, 2,466 developed IHPS in the study period (2.29/1000). Of 4,875 infants prescribed oral azithromycin, 8 developed IHPS (1.64 per 1000). All 8 affected infants were male. The median number of days IHPS developed after AZI prescription was 29.5 d (9-45 d). The relative risk for developing IHPS with AZI exposure during first 90 days of life: 0.71 (95%CI 0.36-1.43; P=0.34. The highest rate of IHPS occurred when AZI exposure was within the first 14 d of life (3/148 (2%); aOR 8.26; 95%CI 2.62-26, P<0.01). In all cases, oral AZI was prescribed for treatment for Chlamydia conjunctivitis or pneumonia and treatment/prophylaxis against Bordetella pertussis. Of note, in a separate report analyzing the association of prematurity and IHPS in the same cohort, there was an increase in IHPS for preterms 33-37 weeks gestation, but not in preterms ≤32 weeks gestation⁹⁶. This study did not include data on inpatient macrolide exposures and did not address IV azithromycin. Due to immaturity of intestinal motilin receptors, oral erythromycin has little effect on intestinal motility in preterm infants <32 weeks gestation and intravenous erythromycin does not affect intestinal motility in preterms⁹⁷. So it is unlikely that either oral or IV azithromycin will affect intestinal function and risk for IHPS in the very preterm study population.

Azithromycin and the risk of potentially fatal heart rhythms: Azithromycin is proarrhythmic with prior reports of occurrences of QT-interval prolongation, and torsades de pointes⁹⁸. Recently, a retrospective study of a large Tennessee Medicaid cohort detected a small absolute increased risk of cardiovascular death (hazard ratio, 2.88; 95% CI, 1.79-4.63) in adults who took a 5 day course of azithromycin compared to individuals who took no antibiotics⁹⁸. There were 47 additional cardiovascular deaths per 1 million azithromycin courses. The increased risk for cardiovascular death was highest among patients with a high baseline risk for cardiovascular disease.

The FDA independently reviewed the study data and released a Drug Safety Communication on 3/12/2013 summarizing their findings and recommendations. The FDA noted important limitations of the study including 1) lack of randomization so patients who received different drugs might have differed in ways that biased the results; 2) study limited to patients treated as outpatients, so patients treated for severe or life-threatening infections were likely not included; 3) cardiovascular deaths were determined by death certificates rather than full medical records; and 4) some limitations to the statistical methods. However, the FDA found that the overall finding of excess risk of cardiovascular death in the azithromycin treated patients was valid and that the excess risk of cardiovascular death, especially of sudden death, is consistent with arrhythmias from drug-related QT prolongation. In addition the FDA evaluated the results of a clinical QT study conducted by the manufacturer assessing the effects of azithromycin on the QT interval in adults. In a randomized, placebo-controlled parallel trial, 116 healthy subjects received either chloroquine (1000 mg) alone or in combination with azithromycin (500 mg, 1000 mg, and 1500 mg once daily). In comparison to chloroquine alone, the maximum mean (95% upper confidence bound) increases in QTcF were 5 (10) ms, 7 (12) ms and 9 (14) ms with the co-administration of 500 mg, 1000 mg, and 1500 mg azithromycin, respectively (see attached Drug label, p. 7), indicating that co-administration of azithromycin increases the QTc in a dose- and concentration-dependent manner. The FDA identified the following groups at higher risk of torsades de pointes and fatal arrhythmia:

- ☐ Patients with known prolongation of the QT interval, a history of torsades de pointes, congenital long QT syndrome, bradyarrhythmias, or uncompensated heart failure
- ☐ Patients on drugs known to prolong the QT interval
- ☐ Patients with ongoing proarrhythmic conditions such as uncorrected hypokalemia or hypomagnesemia, clinically significant bradycardia, and in patients receiving Class IA (quinidine, procainamide) or Class III (dofetilide, amiodarone, sotalol) antiarrhythmic agents

The implications of this study for azithromycin use in newborns and children are unclear. There is a single case report in the literature of cardiac arrest with suspected prolonged QT in a 9 month old who inadvertently received 50 mg/kg azithromycin intravenously over 20 minutes⁹⁹. Prolonged QT interval is rare in newborns with an incidence of the heritable long QT syndromes estimated between 1 per 3000 and 1 per 5000 births¹⁰⁰. Based on a retrospective study of prolonged QT interval in neonates, Villain and co-workers¹⁰¹ suggest that infants presenting with a QTc<0.5 second normalize their QTc over time while infants with a QTc>0.6 second are at risk for severe arrhythmias and sudden cardiovascular death. Prolonged QTc (>0.45 s) was not detected in any of the 171 preterm infants screened for the 10 mg/kg and 20mg/kg single dose studies or any of the 192 infants screened for the 20 mg/kg x 3d multi-dose study.

There are no known interactions of AZI with other drugs. The potential for these adverse outcomes will be carefully evaluated in our clinical trials of AZI in preterm infants.

Pharmacokinetic/Pharmacodynamic of single and multiple doses of AZI in preterm infants:

We have completed a Phase I open-label, pharmacokinetic (PK) study characterizing the single dose PK, safety, tolerability, and biologic effects of 10 and 20 mg/kg IV AZI in mechanically ventilated 24-28 wk gestation preterm neonates who are at high-risk for

Ureaplasma respiratory tract colonization and BPD. A two-compartment structural model with the clearance and volume of peripheral compartment (V2) allometrically scaled on body weight best described the PK of AZI in preterm neonates. The single 10 mg/kg and 20 mg/kg dose regimens were safe, but did not suppress pulmonary inflammatory responses. The 10 mg/kg single dose was insufficient to eradicate *Ureaplasma*, but there were no treatment failures in the 20 mg/kg group.

Pharmacokinetic simulations indicate that even multiple dose administration of 10 mg/kg AZI would be inadequate to maintain AZI plasma concentrations above the *Ureaplasma* MIC₅₀, while multiple doses of 20 mg/kg might provide a favorable AUC₂₄/MIC₉₀ ratio.

We subsequently completed the AZIP2 trial characterizing the multiple dose PK, safety, and biologic effects of 20 mg/kg x3 days regimen. Fifteen subjects 24-28 wk gestation (7 of whom were *Ureaplasma*-positive pre-dose) were enrolled and received all 3 doses of study drug and all were followed to the primary clinical endpoint of BPD at 36 weeks PMA. The population PK model was refined with the addition of the 15 subjects from the multi-dose study. The best model to describe the PK parameters of AZI in preterm infants based on 40 subjects (10 mg/kg single dose (N=12); 20 mg/kg single dose (N=13); and 20 mg/kg multiple dose (N=15)) is the 2 compartment model with all parameters scaled on body weight. Although the percent relative standard error was low for each PK parameter, the intersubject variability is high due to the small number of subjects in the multiple dose group.

Table 1. Population Pharmacokinetic Parameter Estimates in Preterm Infants (N=40)

Parameter	Estimate (%RSE)	%ISV (%RSE)
CL (L/hr)	0.15 x WT (kg) ^{0.75} (10)	58.1 (25)
V1 (L)	1.88 x WT (kg) (11)	78.2 (41)
Q (L/hr)	1.79 x WT (kg) ^{0.75} (10)	64.3 (37)
V2 (L)	13.00 x WT (kg) (12)	78.1 (30)
Residual Error	28 (24)	---
CL: Clearance V1: Volume of distribution of first compartment V2: Volume of distribution of second compartment RSE: relative standard error ISV: intersubject variability calculated as the square root of the estimated variance of intersubject variability WT: body weight in kilograms		

The mean observed plasma concentration of AZI in the 15 subjects in the multiple dose study (Fig. 1) exceeded the MIC₅₀ of 1 µg/ml for *Ureaplasma* isolates¹⁴. This may explain in part the effective eradication of *Ureaplasma* from the respiratory tract in infants who received 20 mg/kg x 3 day regimen (Fig. 2). There were no follow-up

positive cultures and only one sample that was PCR positive 4-5 days post dose in the *Ureaplasma*-infected group. There was no increase in frequency of events potentially related to azithromycin use such as feeding intolerance, sepsis, or hearing loss with the higher dose. The doses were well-tolerated and there were no adverse events attributed to the drug.

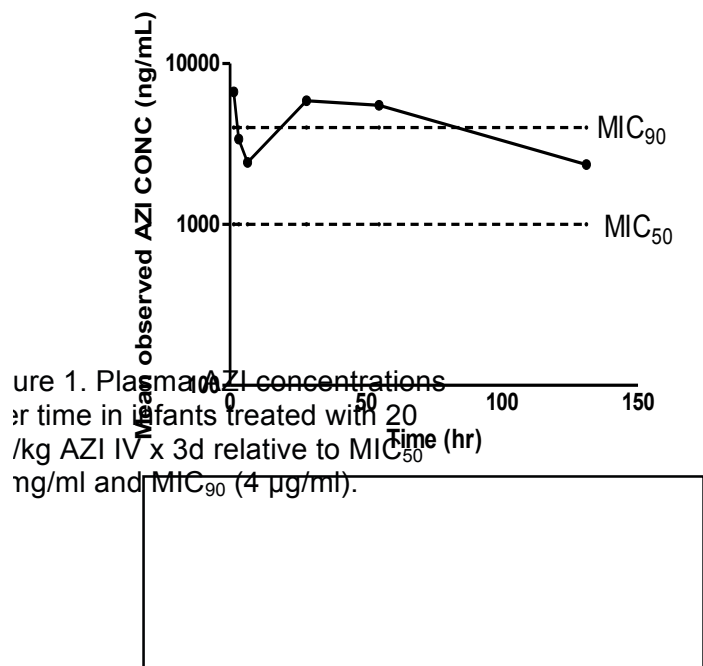


Figure 1. Plasma AZI concentrations over time in infants treated with 20 mg/kg AZI IV x 3d relative to MIC₅₀ (4 µg/ml) and MIC₉₀ (4 µg/ml).

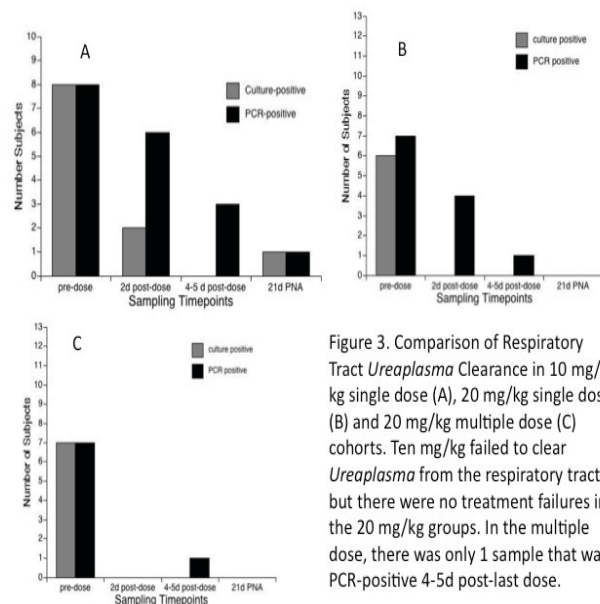


Figure 3. Comparison of Respiratory Tract *Ureaplasma* Clearance in 10 mg/kg single dose (A), 20 mg/kg single dose (B) and 20 mg/kg multiple dose (C) cohorts. Ten mg/kg failed to clear *Ureaplasma* from the respiratory tract, but there were no treatment failures in the 20 mg/kg groups. In the multiple dose, there was only 1 sample that was PCR-positive 4-5d post-last dose.

2.2 Study Rationale

Although not all epidemiologic studies linking *Ureaplasma* spp. respiratory tract colonization with BPD evaluated the duration of colonization^{2,3}, Catro-Alcaraz *et al.*¹⁰² observed that persistent, not transient respiratory colonization increased the risk for BPD at 36 wk PMA. In their cohort, transient colonization occurred in 35% while persistent colonization occurred in 45%. Late acquisition was found in 20%. In a recent prospectively recruited cohort of 112 infants <32 wk gestation with serial respiratory cultures, we observed transient colonization (defined as 1-2 positive cultures in the first wk followed by 1 or more negative cultures > 1 wk of age) rate of 25%. It is likely that persistently positive serial cultures represent true infection and/or ineffective immune response while transient detection represents colonization. To determine the microbiologic efficacy of IV azithromycin to eradicate *Ureaplasma* from the preterm lung, we will conduct a multicenter, randomized, double-blind, placebo-controlled clinical trial of azithromycin 20 mg/kg x 3 days. This endpoint is important to plan for a Phase III clinical trial to determine if ureaplasma eradication improves short-term (BPD) and long-term pulmonary outcomes.

From 11/2013 to 12/2016, 94 subjects have been enrolled in this Phase IIb trial. Initially, 2 GA strata (24⁰-26⁶ and 27⁰-28⁶ wk) were included, but the *Ureaplasma* respiratory colonization rate was 16% in the higher GA stratum and 54% in the lower GA stratum. To limit enrollment to the GA stratum with the highest *Ureaplasma* respiratory colonization rate and to reduce the exposure of non-colonized infants to the potential risks associated with azithromycin, eligibility criteria will be changed in January 2016 to limit the study population to infants 24⁰-26⁶ wk gestation who are at highest risk for *Ureaplasma* respiratory tract colonization and BPD. We will utilize the UAB Diagnostic Mycoplasma Laboratory as a central reference laboratory for *Ureaplasma* culture, genotyping, and azithromycin susceptibility testing. Therefore, infants will receive study drug before *Ureaplasma* colonization status is known. This approach is justified since

the antibiotic will be most beneficial if given early; non-colonized infants may benefit from the anti-inflammatory effects of azithromycin.

3. STUDY DESIGN

The study design will be a double-blind, placebo-controlled clinical trial to test the efficacy and safety of azithromycin 20 mg/kg IV x 3 days to eradicate *Ureaplasma* spp from the respiratory tract of preterm infants 24⁰ to 26⁶ weeks gestation (after January 2016; 24⁰-28⁶ wk GA January 2016 or earlier) exposed to positive pressure ventilation. A total of 140 infants will be randomized to either azithromycin (N=70) or placebo (N=70) stratified by center. The primary outcome will be survival with microbiological eradication of *Ureaplasma* defined as survival to discharge or transfer with 3 negative cultures obtained post-therapy. Secondary outcomes will include physiologic BPD at 36 weeks post-menstrual age (PMA), overall mortality, incidence of co-morbidities of prematurity such as intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, bacterial and fungal nosocomial infection, pulmonary air leak, patent ductus arteriosus, retinopathy of prematurity, number of days of positive pressure ventilation, number of days of oxygen supplementation, use of postnatal steroids, and use of non-study antibiotics. Subjects will be enrolled in 7 level III NICUs (3 sites in Baltimore, MD (University of Maryland Medical Center, Mercy Medical Center, Johns Hopkins Hospital); Christiana Care Health Services, Newark, DE; University of Virginia, Charlottesville, VA; University of Alabama at Birmingham, Birmingham, AL; and Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, TN). Subjects will be followed to 22-26 months adjusted age. The University of Maryland will provide data management and laboratory coordination. The Diagnostic Mycoplasma Laboratory under the direction of Dr. Ken Waites will serve as the central laboratory for all *Ureaplasma* culture, PCR, and antibiotic susceptibility testing.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

The expected race/ethnicity distribution of subjects from the 7 sites is 52% white, 45% African-American, 3% Asian, and 12% Hispanic/ Latino.

4.1 Inclusion Criteria

- Gestational age 24⁰-26⁶ weeks by best obstetrical estimate
- <72 h age
- Positive pressure ventilation for at least 1 hour duration during the first 72 hours of life
- Presence of indwelling intravenous line for drug administration

All participants must meet all of the inclusion criteria to participate in this study.

4.2 Exclusion Criteria

- Any patient judged to be non-viable or for whom withdrawal of life support is planned
- Patients with major lethal congenital anomalies
- Triplets or higher order multiples
- Patients delivered for maternal indications (low risk of *Ureaplasma* colonization)

- Patients with electrocardiogram QT interval corrected for heart rate (QTc) \geq 450 ms
- Patients with significant hepatic impairment (direct bilirubin >1.5 mg/dL)
- Patients exposed to other systemic macrolide
- Patients with clinically suspected *Ureaplasma* CNS infection or other **confirmed** bacterial/viral infection
- Patients participating in other clinical trials involving investigational products.

All screened infants meeting any of the exclusion criteria at baseline will be excluded from study participation.

4.3 Study Enrollment Procedures

All admissions to the NICUs of the 7 clinical sites <72 h age with gestational age 24^0 to 26^6 wk (after January 2016; 24^0 - 28^6 wk GA January 2016 or earlier) will be screened daily by research staff for study eligibility and parental consent of eligible subjects will be obtained (Procedure Schedule, Appendix B). All admissions <72 h age and 24^0 to 26^6 weeks gestation by best obstetric estimate will be further screened for eligibility. For each screened potential subject, an eligibility checklist (CRF AZIP01) will be completed and entry added to a screening log with site-specific numeric patient identification number (PID) and letter code (LETCODE) (Sample Screening Log, Appendix A). The screening log with patient identifiers is maintained at each site in a secure location and is not submitted to the Data Coordinating Center. As part of the screening process, an electrocardiogram (EKG) QTc interval will be calculated from lead II from a potential subject's monitor and a copy of rhythm strip and QTc calculation submitted on CRF AZIP02 for enrolled subject.

If the answer to all inclusion criteria is yes and answer to all exclusion criteria is no, study personnel will approach parent(s) to discuss the research study and request informed consent. If a potential subject is eligible, but consent is not obtained, a reason (e.g. missed, maternal age <18 , parent/LAR refused consent, non-English speaking parent/LAR) will be required on the submitted eligibility checklist CRF (AZIP01). If an infant qualifies, the PI or coordinator will obtain informed parental consent prior to initiation of study procedures. An EKG for QTc measurement will be obtained after consent if not done previously during the screening process. If the PI is also responsible for the clinical care of a potential subject, he/she will not participate in the consent discussion with the parents to avoid undue pressure on the parents to agree to the study. The information provided in the consent will cover the elements listed in the CFR Part 50.25 and be approved by the site Institutional Review Board (IRB). This includes the investigational nature and objectives of the trial; the procedures and treatments involved and their attendant risks, discomforts, and benefits; and the potential alternative therapies, alternative to not participate and right to withdraw without penalty will be explained. The study staff personnel will offer to answer any questions.

In the consent process, banking (storage) of *Ureaplasma* samples isolated from positive subjects will be explained to parents. They can decline banking of their child's specimens and still participate in this study. They can indicate on the consent form whether they would be willing or not to be contacted for future studies of their child's banked specimens. For each question (banking vs no banking; future contact or not), the parent will initial their choice.

If a parent consents to their child's participation, the parent will sign and date the consent form. One copy of the signed consent form will be provided to the parents, another copy will be placed in the child's medical record and the original signed consent form will be kept in a secure file at each site. Written documentation of the consent process will be placed in the medical record. The subject will be assigned a study identification number. The study identification number will be recorded on all study forms. (See Section 16.1 for model consent form).

4.3.1 Randomization procedure:

There will be separate randomization schedules for each center. Infants will be randomized according to a permuted-block design to receive azithromycin or placebo. Twins will be randomized together to the same study arm. Randomization schedules prepared by the UMB biostatistician will be provided to the AZIP Randomization Center located at Axio Research (Seattle, WA) that will establish a web-based randomization site available 24 hours/day and seven days a week. Separate randomization schedules for each stratum within each clinical site will be prepared. The randomizations on the schedules will be in varying block sizes of two, four and six to force approximate balance of azithromycin and placebo treatment assignments at each clinical site. The University of Maryland Investigational Drug Service (UMB Coordinating Pharmacy) will receive the randomization number assignments for each site to prepare study drug shipments. The UMB Coordinating Pharmacy will ship to the study pharmacist at each site numbered study drug kits for active drug and sealed envelopes each labeled with a site-specific randomization number and containing a label for "azithromycin" or "placebo". Each randomization is documented for time of issue on the computer system and under whose user ID authority the allocation is issued (i.e., who requested the randomization). Once a randomization number is assigned and study pharmacist is notified, he/she will open the corresponding envelope, prepare the appropriate drug or placebo, and attach the label from the opened envelope on the drug accountability log. For study drug, one vial per dose will be reconstituted with D5W to final concentration of 2 mg/ml. For placebo, an equivalent volume of D5W will be prepared. Axio Research will cooperate with the UMB Coordinating Pharmacy in kit preparation, labeling and shipping to maintain an adequate inventory at each clinical site. In the event of enrollment of twins, the system will specify two numbers containing the same, blinded treatment. Axio Research randomization application will provide unblinded reports to assist UMB Coordinating Pharmacy staff in maintaining appropriate levels of drug kits at each site. A customized web page will allow the unblinded pharmacist to indicate to the application which kits have been sent to and received by the clinical sites (i.e., available for dispensing at randomization).

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

5.1.1 Drug Dose Selection Rationale

We have selected a multiple dose for evaluation in this proposal based on the population PK model that includes the 10 mg/kg single dose (N=12) and 20 mg/kg single (N=13) and multiple dose (N=15) PK, and the clinical experience in older children with respiratory tract infections. The PK properties of AZI support short courses of therapy. The drug has a long elimination half-life (>50 h), is concentrated in phagocytic cells with good tissue penetration, especially in lung, and persists in tissues for much

longer than in plasma¹⁰³. Pharmacodynamic studies suggest that it is the AUC_{24(u)}/MIC₉₀ ratio that is most predictive of AZI efficacy¹⁰⁴. Increasing the dose is more effective than increasing duration of treatment for increasing the AUC₂₄/MIC₉₀ ratio. Results of experimental infections in animal models suggest that increasing the dose early in the infectious process will result in clearance of even marginally susceptible organisms¹⁰⁵.

Although optimal AUC_{24(u)}/MIC₉₀ ratios for AZI to eradicate other common organisms were reported to range from 0.5 to 14.8 h¹⁰⁶, the optimal AUC_{24(u)}/MIC₉₀ for AZI to effectively eradicate *Ureaplasma* is still unknown. Based on our recent clinical study in preterm neonates, a dosage regimen of 20 mg/kg/d x 3d IV AZI was deemed safe and effective and resulted in an AUC_{24(u)}/MIC₉₀ ratio of 5.69 as indicated in Table 2.

Table 2. AUC₂₄ and AUC₂₄/MIC₉₀ for potential dose regimens for *Ureaplasma* therapy.

Dose Regimen	AUC _{24(u)} ^{**} (µg*h/ml)	AUC _{24(u)} /MIC ₉₀ (h)
10 mg/kg/d x 1d (observed)	12.00	3.00
20 mg/kg/d x 1d (observed)	21	5.25
20 mg/kg/d x 3d (observed)	22.8	5.69
^{**} assuming 30% of AZI dose is bound to plasma proteins and MIC ₉₀ for AZI against <i>Ureaplasma</i> is 4 µg/ml		

As such, we predict that an AUC_{24(u)}/MIC₉₀ ratio of > 5.69 h is needed in order for AZI dosage regimen to eradicate *ureaplasma* in preterm neonates.

The data from the 20 mg/kg multiple dose Phase IIa study confirmed the simulations and *Ureaplasma* was eradicated from the respiratory tract of all subjects colonized pre-dose. The proposed short course will reduce development of drug resistance and limit the risk for increased mortality

and necrotizing enterocolitis (NEC) recently reported for preterm infants exposed to a prolonged duration of initial empirical antibiotic treatment¹⁰⁷. The 3 day 20 mg/kg oral AZI regimen is well tolerated in older children⁹³, and is more effective in treating recurrent or persistent otitis media than high dose amoxicillin-clavulanate¹⁰⁸ and group A streptococcal tonsillopharyngitis than 3 d 10 mg/kg dose oral AZI or 10 d penicillin V^{109,110}.

5.1.2 Drug Dosage, Administration, and Schedule

After baseline safety laboratory tests and 2 tracheal aspirate samples at least 2 h apart and one nasopharyngeal sample for *Ureaplasma* culture and PCR from intubated infants, or 2 NP samples at least 2h apart from non-intubated infants have been obtained, study infants will receive according to randomization assignment, either azithromycin administered at a concentration of 2 mg/ml in D5W or placebo of same volume D5W as a single intravenous infusion over 60 ± 5 min using a functioning intravenous line within 24 h of enrollment. The dose will be calculated as 20 mg/kg actual body weight. Each subject's actual body weight at the time of study entry will be used for determining the injection volume of study drug. For study drug, one vial per dose will be reconstituted with D5W to final concentration of 2 mg/ml. For placebo, an equivalent volume of D5W will be prepared. A total of 3 doses will be administered at 24 ± 0.5 h intervals. A pharmacist from the Investigational Drug Service Pharmacy for each site will be responsible for dispensing and accounting for study drug. A NICU nurse will administer the study drug.

5.1.3 Duration of Subject Participation

Enrolled subjects will receive 3 doses of azithromycin or placebo and will be followed for safety until discharge or transfer or *44 weeks post-menstrual age (PMA), whichever comes first*. Respiratory cultures for *Ureaplasma* will be obtained pre-dose and 2d, 4-5 d post *last* dose and 21 d of age. A room air challenge test will be performed when the infant is 36 ± 1 weeks post-menstrual age or at discharge if earlier. A respiratory outcome questionnaire will be administered at 6, 12, and 22-26 months corrected age by phone or in-person interview. A comprehensive neurodevelopmental assessment will be performed at 22-26 months adjusted age.

5.1.4 Stopping Rules for Individual Subjects

Discontinuation Infusion of Study Drug: Study drug administration will be delayed or discontinued if any subject has evidence of an arrhythmia on the cardiac monitor (they will all have continuous cardiopulmonary monitoring) or an ongoing proarrhythmic condition such as hypokalemia (serum potassium < 3.5 mEq/L), hyperkalemia (serum potassium > 6.5 mEq/L on non-hemolyzed specimen), hypomagnesemia (total serum Magnesium ≤ 1.6 mg/dL), hypocalcemia (ionized calcium < 3.0 mg/dL (< 0.75 mmol/L)) or hypothermia (core temperature $\leq 35^{\circ}\text{C}$) until corrected by the clinical care team. Infusion of study drug will be discontinued for any of the following changes in vital signs unless it can be remedied by a simple maneuver (e. g., correcting the patency of the airway, reestablishment of artificial airway, simple stimulation for apnea of prematurity, etc.): Decrease in BP that requires fluid bolus or pharmacologic support, or *sustained* HR less than 80 bpm. If Study Drug is permanently discontinued due to an AE, including the above, this will be recorded on the AE CRF and the subject continued to be followed until discharge or transfer.

5.1.5 Plans for Study Interruption Based on Safety Monitoring

The DSMB members will review all deaths and all adverse reactions or suspected adverse reactions and make recommendations concerning continuation of the trial. The sites are required to submit reports of all adverse events that are serious and unexpected regardless of relationship to study drug within 5 business days to the sponsor. The sponsor will forward all reports to Dr. Agthe the clinical monitor for review of possible relationship to drug and to the DSMB chair. The DSMB chair may request treatment assignment after reviewing the initial report or at the time of the monthly summary report. A summary of cumulative serious adverse events by study arm will be provided by the Data Coordinating Center to the DSMB chair monthly. The DSMB will meet to review safety data after 50% enrollment. The DSMB recommended that there be no pre-specified stopping rules for the study, but rather agreed to review SAEs as they occur. The DSMB chair may call a DSMB meeting to specifically review safety concerns. The DSMB recommendations may include study termination, study continuation with protocol modifications (e.g., change in eligibility criteria), or temporary suspension of enrollment until further evaluation is completed. Per DSMB recommendations, any death that occurs within 30 d of drug administration will be reviewed by the DSMB with each death occurrence.

5.1.6 Dose Adjustments

No dose adjustments will be made in this study. If the existing IV line stops functioning during the study drug infusion, the remainder of the drug infusion will be given via a second IV site. If all IV access is lost during the study drug infusion, the amount infused will be recorded and the remainder of study drug returned to the investigational study pharmacy.

5.2 Handling of Study Interventions

5.2.1 Packaging and Formulation

Azithromycin will be manufactured and packaged by American Pharmaceuticals Partners (Schaumburg, IL) and shipped using appropriate clinical trial drug distribution procedures. Azithromycin for injection will be supplied in lyophilized form under a vacuum in a 10 ml vial equivalent to 500 mg of azithromycin for intravenous administration. For study drug, one vial per dose will be reconstituted with D5W to final concentration of 2 mg/ml. An initial solution of 100 mg/ml azithromycin for injection will be prepared by adding 4.8 ml of sterile water for injection to the 500 mg vial and shaking the vial until all of the drug is dissolved. To provide the azithromycin in the final concentration of 2 mg/ml, 1 ml (100 mg) of the reconstituted drug will be transferred to a syringe containing 49 ml dextrose 5% injection and the syringe will be inverted several times to mix completely. When diluted in this manner, azithromycin for injection is stable for 24 hrs at or below room temperature (30°C or 86°F) or for 7 days, if stored under refrigeration (5°C or 41°F). Placebo will be an equal volume of D5W.

5.2.2 Labeling

Information presented in the label for study medication will comply with hospital investigational pharmacy and local regulatory requirements.

5.2.3 Storage

The reconstituted azithromycin will be stored in a refrigerator. The reconstituted drug is stable for 7 days if stored under refrigeration (5°C or 41°F). For the study, one vial per dose will be prepared. Vials of azithromycin or placebo will not be used if any particulate matter or discoloration is observed. Records of the actual storage conditions during the period of the study will be maintained.

5.2.4 Preparation

The azithromycin will be supplied in lyophilized form under a vacuum in a 10 ml vial equivalent to 500 mg of azithromycin for intravenous administration. Each vial also contains sodium hydroxide and citric acid. To prepare each dose of azithromycin, using aseptic technique, 4.8 ml of sterile water for injection will be added to the 500 mg vial and mixed by shaking the vial until all drug is dissolved. Each mL of reconstituted solution will contain 100 mg azithromycin. This solution is stable for 24 hours when stored below 30°C or 86°F. To provide azithromycin in the final concentration of 2 mg/ml, 1 ml of the 100 mg/ml azithromycin solution will be added to 49 ml dextrose 5% injection ml of 5% dextrose in water in a syringe. The patient specific dose of azithromycin 2 mg/ml is drawn up and dispensed as a syringe to be infused intravenously over 60±5 min. If the subject's weight is ≤ 0.94 kg, the dose may be ordered to the 1/10 of 1 ml using a 10 ml syringe for administration with a syringe pump (example $0.94 \text{ kg} \times 10 \text{ ml/kg} = 9.4 \text{ ml}$ of 2 mg/ml azithromycin or equal volume of D5W). For subjects >0.94 kg, a 20 ml syringe with 1ml gradations will be required, and the ordered drug volume should be rounded to nearest ml (example subject weight $1.23 \text{ kg} \times 10 \text{ ml/kg} = 12.3 \text{ ml}$, so 12 ml should be ordered). For placebo, an equivalent volume of D5W will be administered IV over 60±5 min.

5.2.5 Supply and Return of Drug

The University of Maryland Medical Center (UMMC) Investigational Drug Service will be Pharmacy Coordinating Center for the study. Azithromycin study drug kits will be packaged, labeled, and distributed to the study site pharmacies by the UMMC

Coordinating Pharmacy. Only 1 study drug kit will be used for each enrolled subject; each kit will have a unique number. At study initiation, and as needed thereafter, azithromycin kits will be shipped to a responsible person (e.g. pharmacist) at the Investigational Drug Service of each participating center, who will check the amount and condition of the study drug and enter this data into a Proof of Receipt form and Investigational Product Accountability Record (see next section). The Proof of Receipt form should then be faxed to the UMMC Coordinating Pharmacy. The Investigational Drug Pharmacy will record the final disposition of all study drug.

5.2.6 Drug Accountability

The study pharmacist at each site will submit monthly accountability logs to the UMB Coordinating Pharmacy. The PI Dr. Viscardi is responsible for the accountability of all used and unused trial supplies. The following information will be recorded:

- a. The batch number, dates and quantities of the drug received from supplier
- b. Subject's identification (subject number, letter code, and study drug number)
- c. Date and quantity of drug dispensed
- d. The initials of the dispensing individual
- e. Date and quantity of drug returned to the investigator/pharmacy

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

All medications except other macrolide antibiotics are allowed as clinically prescribed.

5.3.2 Required Interventions None

5.3.3 Prohibited Interventions: Any other macrolide antibiotic (e.g. erythromycin, clarithromycin, clinically prescribed azithromycin)

5.4 Adherence Assessment

NICU medical staff will be responsible for administering all doses of study drug and recording the exact times of the initiation and completion of drug infusion in the CRF. If study drug administration is incomplete or discontinued, the reason will be provided on the drug administration CRF and a protocol deviation report will be submitted.

6.0 Study Schedule

6.1 Study Schedule

6.2 Description of Evaluations

6.2.1 Screening Evaluation

All infants admitted less than 72 h of age with gestational age 24^0 to 26^6 weeks gestation (after January 2016; 24^0 - 28^6 wk GA January 2016 or earlier) by best obstetric estimate will be screened for eligibility. A QTc interval will be calculated from the lead II of the heart rate monitor either before consent procedure or after consent is obtained, but before any other study procedures commence. If the QTc is >450 ms, the subject is ineligible for participation in the study. For each screened potential subject, an eligibility checklist (CRF AZIP01) will be completed and entry added to a screening log with site-specific numeric patient identification number (PID) and letter code (LETCODE). The screening log with patient identifiers is maintained at each site in a secure location and is not submitted to the Data Coordinating Center.

6.2.2 Consenting Procedure

If the answer to all inclusion criteria is yes and answer to all exclusion criteria is no, study personnel will approach parent(s) to discuss the research study and request informed consent. If a potential subject is eligible, but consent is not obtained, a reason (e.g. missed, maternal age <18 , parent/LAR refused consent, non-English speaking parent/LAR) will be required on the submitted eligibility checklist CRF (AZIP01). If an infant qualifies, the PI or coordinator will obtain informed parental consent prior to initiation of study procedures. An EKG for QTc measurement will be obtained after consent if not done previously during the screening process. If the PI is also responsible for the clinical care of a potential subject, he/she will not participate in the consent discussion with the parents to avoid undue pressure on the parents to agree to the study. The information provided in the consent will cover the elements listed in the CFR Part 50.25 and be approved by the site Institutional Review Board (IRB). This includes the investigational nature and objectives of the trial; the procedures and treatments involved and their attendant risks, discomforts, and benefits; and the potential alternative therapies, alternative to not participate and right to withdraw without penalty will be explained. The study staff personnel will offer to answer any questions.

In the consent process, banking (storage) of *Ureaplasma* samples isolated from positive subjects will be explained to parents. They can decline banking of their child's specimens and still participate in this study. They can indicate on the consent form whether they would be willing or not to be contacted for future studies of their child's banked specimens. For each question (banking vs no banking; future contact or not), the parent will initial their choice.

If a parent consents to their child's participation, the parent will sign and date the consent form. One copy of the signed consent form will be provided to the parents, another copy will be placed in the child's medical record and the original signed consent form will be kept in a secure file at each site. Written documentation of the consent process will be placed in the medical record. The subject will be assigned a study identification number. The study identification number will be recorded on all study forms. (See Section 16.1 for model consent form).

6.2.3 Screening

All screening evaluations, completion of eligibility checklist, and informed consent must be obtained within 72 h of birth. All screened infants must meet all of the inclusion criteria to participate in this study. All screened infants meeting any of the exclusion criteria at baseline will be excluded from study participation.

Specific inclusion criteria to be determined:

- *Gestational age 24⁰-26⁶ weeks (after January 2016; 24⁰-28⁶ wk GA January 2016 or earlier) by best obstetric estimate:* Infant must be between 24 weeks 0 days and 26 weeks and 6 days gestation to be eligible for the study. The gestational age estimate will be based on a hierarchy of the available information with the order from most to least accurate estimate (date of intrauterine insemination > fetal ultrasound before the 14th week; fetal ultrasound at 14 or more weeks (if no earlier ultrasounds) > last menstrual period (LMP) without fetal ultrasound > gestational age by Ballard exam in absence of fetal ultrasounds or LMP).
- *Positive pressure ventilation for at least 1 h duration during the first 72 h of life.* This is defined as receipt of positive pressure by intubation and mechanical ventilation or nCPAP for at least 1 continuous hour. The criterion will be met if the hour of positive pressure is by one of the modes of ventilation (mechanical ventilation or nCPAP) or combination (e.g. intubation for exogenous surfactant administration followed by extubation to nCPAP).
- *Presence of indwelling intravenous line for drug administration*

Specific exclusion criteria to be determined:

- *Any patient judged to be non-viable or for whom withdrawal of life support is planned.* **Viable**, as it pertains to the neonate, means being able, after delivery, to survive (given the benefit of available medical therapy) to the point of independently maintaining heartbeat and respiration, *in the opinion of the attending neonatologist*. Neonates for whom death is considered imminent or are unresponsive to medical cardio-respiratory support defined as persistent hypoxemia ($\text{PaO}_2 < 40$), hypercarbia ($\text{PaCO}_2 > 80$) and acidosis ($\text{pH} < 7.0$) for >12 h will be considered non-viable for study criteria.
- *Major lethal congenital anomalies:* Those anomalies that will likely result in death such as renal agenesis, pulmonary hypoplasia, cyanotic congenital heart disease.
- *Triplets or higher order multiples* are excluded
- *Patients delivered for maternal indications.* Maternal indications include, but are not limited to pre-eclampsia, eclampsia, pulmonary hypertension, trauma, or malignancy. *Patients whose mothers have these conditions (e.g. pre-eclampsia, chronic hypertension), but are delivered for fetal indications such as preterm labor or premature preterm rupture of the membranes are eligible.*
- *Patients with significant hepatic impairment (Direct Bilirubin >1.5 mg/dL):* A fractionated bilirubin should be obtained and documented in medical record prior to enrollment. If a fractionated bilirubin is not available during screening prior to consent, then informed parental consent can be obtained first and a fractionated bilirubin obtained prior to enrollment and randomization.
- *Exposure to other systemic macrolide:* Prior exposure of the infant to systemic erythromycin, clarithromycin, telithromycin, other macrolides, or clinically ordered azithromycin is an exclusion criterion. Exposure to ophthalmologic erythromycin ointment is not considered a systemic exposure.

- *Participation in other clinical trials involving investigational products.* Participation in other clinical trials of investigative drugs is an exclusion criterion. Participation in clinical research studies not involving investigational product is allowed.
- *EKG QTc \geq 450 ms.* Measure EKG QTc interval (See CRF AZIP 02):

Calculation of EKG QTc (average 3 measurements):

0. Print strip of lead II from potential subject monitor (paste copy to page)
1. May calculate manually or use http://en.ecgpedia.org/wiki/QTc_Calculator
2. Measure QT interval (duration from the beginning of the QRS complex to the end of the T wave): count the number of small squares, then multiply by 0.04 seconds(s) x 1000: _____ ms
3. Measure RR interval (interval from the peak of one R wave to next R wave peak), count the number of small squares, then multiply by 0.04 seconds(s): _____
4. $QTc = QT(ms) / \sqrt{RR(s)}$
5. Is the Qtc \geq 450 ms? _____ Yes _____ No

The EKG can be completed during screening or after consent is obtained, but prior to any study procedures.

6.3 Enrollment, Baseline, and/or Randomization

6.3.1 Enrollment

The enrollment date is the date *and time* all screening criteria have been met and the parent signed the consent form. Baseline assessments, including tracheal and NP aspirates, randomization, and the first dose of study drug should be initiated within 24 h of obtaining informed consent.

6.3.2 Baseline Assessments

- Demographics, obstetric history, maternal macrolide exposure, neonatal characteristics including respiratory support mode and supplemental oxygen at study entry
- Morbidities of prematurity: Morbidities associated with prematurity are protocol-specified potential serious adverse events. These are events that are anticipated to occur in the preterm population even in the absence of drug exposure. All morbidities present from the time of birth up to the start of Study day 1 (first dose study drug infusion) will be recorded.
- Clinical laboratory tests: Values for electrolytes, total/direct bilirubin, CBC with differential cell count, and platelets obtained within 24 h pre-first dose will be recorded.
- Physical examination: The site PI or other physician listed on local protocol and FDA1572 form will complete the physical assessment and complete section on CRF AZIP 07.
- Tracheal and nasopharyngeal aspirates: For subjects who are intubated at time of enrollment, 2 tracheal aspirates (first for cytokine sample and second for *Ureaplasma* culture with separate suction catheters) will be obtained. The first NP sample will be obtained for culture at the time of first tracheal aspirate. At least 2 hours after the first

suction episode, a second culture sample will be obtained from the ET tube if subject is still intubated and from the nose if subject is extubated. For subjects who are extubated at the time of enrollment, 2 NP samples will be obtained at least 2 h apart for culture only. A TA sample may be obtained for the second sample if infant is intubated at time of second sampling.

6.3.3 Randomization

The enrolled subject will be randomized once all screening procedures are completed and parental informed consent obtained. Randomization should occur within 22 h of enrollment to allow sufficient time for investigational pharmacy to prepare the study drug. The first dose of study drug should be infused no later than 24 h after enrollment.

6.3.4 Blinding

The study pharmacists will be unblinded, but will not reveal treatment assignment to study staff administering the study drug. In the (unexpected) event of a clinically urgent reason for unblinding of a subject's assignment, the clinical site investigator or any other individual will be directed in the kit instructions to contact (in order of preference) Dr. Viscardi, Dr. Terrin, the UMB Coordinating Pharmacy, or Axio Research to document the reasons for unblinding, assure that there is truly compelling cause to unblind, and obtain the treatment information which will be available from all four sources. Date and time of any unblinding as well as reasons for unblinding will be documented by the source providing the unblinding information. Because of the nature of azithromycin and the restriction of the patient population to experienced NICUs, we expect that there will be few or no unblinding events in this study.

6.3.5 Follow-up Visits

A total of 3 doses of study drug will be administered at 24 ± 0.5 h intervals. Follow-up tracheal/NP aspirates will be obtained 2 and 4-5 d post last dose and on postnatal day 21. Safety and tolerance will include vital signs (temperature, pulse, respiratory rate, blood pressure), frequency of apnea (cessation of breathing for > 20 seconds), frequency of bradycardia (heart rate < 80 beats per minute for > 10 sec), cardiac rhythm, pulse oximetry, clinical laboratory testing and Adverse Events (AE). Safety assessment for subject vital signs, frequency of apnea and bradycardia episodes, pulse oximetry, and cardiac rhythm will be collected 1 and 4 h after the initiation of azithromycin infusion. On study day 3 (± 1 d), 7 (± 2 d), 14 (± 2 d) and every 2 wks (± 2 d) until discharge, transfer, or 44 weeks PMA, whichever occurs first, subjects will be assessed for morbidities associated with prematurity, and adverse events. Clinically relevant AEs that will be specifically evaluated include: local site reaction including phlebitis, gastrointestinal signs (abdominal distension, gastric aspirates, emesis, heme-positive stools), intestinal obstruction including radiographic-confirmed pyloric stenosis, culture-proven sepsis with other bacterial or nonbacterial pathogens, necrotizing enterocolitis (NEC), and cardiac arrhythmias. We will also specifically record adverse events that may signal potential proarrhythmic effects in our study population such as sudden death, ventricular tachycardia, ventricular fibrillation and flutter, and seizures. A hearing screening test as part of clinical care will be performed before discharge. Electrocardiograms will be performed pre-dose in all subjects and post-dose if a cardiac arrhythmia is noted on the patient continuous monitor. Clinical laboratory testing (complete blood count with manual differential, electrolytes, liver function, and renal function) will be performed during the 24 hours pre- first dose, and study day 3 (± 1 d), and 7 (± 2 d), 14 (± 3 d), and 28 (± 3 d). Blood draws for study only labs

will be minimized by utilizing lab values from clinically indicated lab draws within the sampling periods. Relevant systemic medications received by subjects during the 7 days post-first dose administration will be recorded.

Table 4. Timing of endotracheal and nasopharyngeal cultures

Samples	Pre-infusion Day 0	2 Day Post-last dose	4-5 Day Post-last dose	postnatal d21 of age
TA culture/PCR	X (2)	X	X	X
NP culture/ PCR	X	X*	X*	X*
TA cytokines	X	X	X	X
Collect only in the event that the infant is not intubated and a TA is not available				

- Study D1 (first drug dose):
 - **Vital Signs:** Vital signs (HR, RR, and oxygen saturation (SpO₂)) will be obtained within 1 h pre-dose, within 20 minutes post-dose and 2h ± 20 min and 4h ± 20 min post-dose
 - **Treatment Administration:** The investigational pharmacy at each site will prepare study drug vial as assigned by randomization of final concentration of 2 mg/ml and will dispense study drug for ordered volume to provide 20 mg/kg. The subject will receive azithromycin administered as a single intravenous infusion over 60± 5 min using a functioning intravenous line within 24 h of enrollment. The dose will be calculated as 20 mg/kg actual body weight. Each subject's actual body weight at the time of study entry will be used for determining the injection volume of study drug. A NICU nurse will administer the study drug.
 - **Concomitant Medications:** Relevant systemic medications received by subjects during the 7 days post-first dose administration will be recorded.
 - **Adverse Events:** Subjects will be assessed for morbidities associated with prematurity, and adverse events. Clinically relevant AEs that will be specifically evaluated include: local site reaction including phlebitis, gastrointestinal signs (abdominal distension, gastric aspirates, emesis, heme-positive stools), intestinal obstruction including radiographic-confirmed pyloric stenosis, culture-proven sepsis with other bacterial or nonbacterial pathogens, necrotizing enterocolitis (NEC), and cardiac arrhythmias.
- Study D2 (second drug dose):
 - **Vital Signs:** Vital signs (HR, RR, and oxygen saturation (SpO₂)) will be obtained within 1 h pre-dose, within 20 minutes post-dose and 2h ± 20 min and 4h ± 20 min post-dose
 - **Treatment Administration:** The investigational pharmacy at each site will prepare study drug vial as assigned by randomization of final concentration of 2 mg/ml and will dispense study drug for ordered volume to provide 20 mg/kg. If the subject's weight is ≤ 0.94 kg, the dose may be ordered to the 1/10 of 1 ml using a 10 ml syringe for administration with a syringe pump (example 0.94 kg x 10 ml/kg= 9.4 ml of 2 mg/ml)

azithromycin or equal volume of D5W). For subjects >0.94 kg, a 20 ml syringe with 1ml gradations will be required, and the ordered drug volume should be rounded to nearest ml (example subject weight 1.23 kg x 10 ml/kg=12.3 ml, so 12 ml should be ordered). The subject will receive azithromycin administered as a single intravenous infusion over 60± 5 min using a functioning intravenous line within 24 ± 0.5 h of first dose. The dose will be calculated as 20 mg/kg actual body weight. Each subject's actual body weight at the time of study entry will be used for determining the injection volume of study drug. A NICU nurse will administer the study drug.

- *Concomitant Medications:* Relevant systemic medications received by subjects during the 7 days post-first dose administration will be recorded.
- *Adverse Events:* Subjects will be assessed for morbidities associated with prematurity, and adverse events. Clinically relevant AEs that will be specifically evaluated include: local site reaction including phlebitis, gastrointestinal signs (abdominal distension, gastric aspirates, emesis, heme-positive stools), intestinal obstruction including radiographic-confirmed pyloric stenosis, culture-proven sepsis with other bacterial or nonbacterial pathogens, necrotizing enterocolitis (NEC), and cardiac arrhythmias.

- Study D3 (Third drug dose):

- *Vital Signs:* Vital signs (HR, RR, and oxygen saturation (SpO₂)) will be obtained within 1 h pre-dose, within 20 minutes post-dose and 2h ± 20 min and 4h ± 20 min post-dose
- *Treatment Administration:* The investigational pharmacy at each site will prepare study drug vial as assigned by randomization of final concentration of 2 mg/ml and will dispense study drug for ordered volume to provide 20 mg/kg. The subject will receive azithromycin administered as a single intravenous infusion over 60± 5 min using a functioning intravenous line within 24 ± 0.5 h of second dose. The dose will be calculated as 20 mg/kg actual body weight. Each subject's actual body weight at the time of study entry will be used for determining the injection volume of study drug. A NICU nurse will administer the study drug.
- *Concomitant Medications:* Relevant systemic medications received by subjects during the 7 days post-first dose administration will be recorded.
- *Clinical laboratory tests:* Complete blood count with manual differential, electrolytes, liver function, and renal function will be performed, and study day 3 (±1 d).
- *Adverse Events:* Subjects will be assessed for morbidities associated with prematurity, and adverse events. Clinically relevant AEs that will be specifically evaluated include: local site reaction including phlebitis, gastrointestinal signs (abdominal distension, gastric aspirates, emesis, heme-positive stools), intestinal obstruction including radiographic-confirmed pyloric stenosis, culture-proven sepsis with other bacterial or nonbacterial pathogens, necrotizing enterocolitis (NEC), and cardiac arrhythmias.

- Study D5 (2 d post last dose):

- *Respiratory samples:* Tracheal aspirate (intubated subject) or NP aspirate (non-intubated subject) for follow-up *Ureaplasma* culture, PCR, and antibiotic susceptibility testing. Separate tracheal aspirate will be collected for cytokine analysis if subject is still intubated.

- *Concomitant medications*: Relevant systemic medications received by subjects during the 7 days post-first dose administration will be recorded.
- *Adverse Events*: Subjects will be assessed for morbidities associated with prematurity, and adverse events.
- Study D6-7 (4-5 d post last dose):
 - *Respiratory samples*: Tracheal aspirate (intubated subject) or NP aspirate (non-intubated subject) for follow-up *Ureaplasma* culture, PCR, and antibiotic susceptibility testing. Separate tracheal aspirate will be collected for cytokine analysis if subject is still intubated.
 - *Concomitant medications*: Relevant systemic medications received by subjects during the 7 days post-first dose administration will be recorded.
 - *Clinical laboratory tests*: Complete blood count with manual differential, electrolytes, liver function, and renal function will be performed on study day 7 (\pm 2 d).
 - *Adverse Events*: Subjects will be assessed for morbidities associated with prematurity, and adverse events.
- Postnatal Day 21:
 - *Respiratory samples*: Tracheal aspirate (intubated subject) or NP aspirate (non-intubated subject) for follow-up *Ureaplasma* culture, PCR, and antibiotic susceptibility testing. Separate tracheal aspirate will be collected for cytokine analysis if subject is still intubated.
- 36 weeks postmenstrual age/pre-transfer:
 - *BPD assessment*: For neonates at 36 ± 1 week or pre-discharge who are on positive pressure support or receiving $> 30\%$ supplemental oxygen with oxygen saturations between 90% and 96%, the diagnosis of BPD will be assigned with no further testing. For neonates at 36 ± 1 week or pre-discharge who are receiving oxygen $< 30\%$ with oxygen saturations between 90% and 96% or receiving oxygen $\geq 30\%$ with oxygen saturations $>96\%$ will undergo a timed step-wise reduction by 2% increments every 5 minutes to room air and a period of observation in room air for 30 minutes as described by Walsh et al.¹¹¹. For infants receiving supplemental oxygen by nasal cannula, the delivered oxygen concentration or “effective FiO_2 ”¹¹¹ will be calculated by the technique described by Benaron et al.¹¹² that was used in the STOP-ROP trial¹¹³ which is based on weight, oxygen liter flow, and oxygen concentration. Failure of the room air challenge will be defined as oxygen saturations 80-89% for 5 consecutive min or $<80\%$ for 15 s. No BPD will be defined as treatment with room air with oxygen saturation $\geq 90\%$ or passing the timed, oxygen-reduction test. Infants will be continuously monitored and observed throughout the reduction test. Study subjects who are transferred or discharged at ≤ 35 wk on supplemental oxygen will be considered to have BPD.
- Pre-discharge/transfer
 - *Hearing assessment*: A hearing screening test as part of clinical care will be

performed before discharge. Test results will be recorded as passed, refer one ear, or refer both ears. If a brainstem auditory response test is requested for clinical care, the results will be recorded as passed or failed.

- *Cumulative days of respiratory support:* The total number of days of mechanical ventilation, nCPAP, and supplemental oxygen will be recorded.
- *Adverse events:* Subjects will be assessed for morbidities associated with prematurity, and adverse events.
- *Pulmonary Follow-up*
 - *Respiratory questionnaires:* Data will be collected by structured parental interviews before discharge and follow-up phone interview at 6, 12 and 22-26 months adjusted age. With permission, we will utilize the validated Tucson Children's Respiratory Study questionnaires that were designed to elicit a complete history of possible covariates such as family history of asthma or atopy, and important environmental (e.g., smoking) and infectious exposures and detailed interval respiratory health history. Recurrent wheezing and chronic cough will be defined as occurring more than twice per week.
 - *The ideal times of visits and points of reference for data collection on long term pulmonary outcomes are six months, one year and two years adjusted age. To assure that patient contact information can be used regardless of dates of actual contact with the babies and their families, we have established contiguous windows for the three follow-up visits. The first of these follow-up visit windows extends from five months to nine months adjusted age with an ideal visit date at six months adjusted age; the second window from 10 months to 18 months with an ideal visit date at 12 months; and the third from 19 months to 30 months with the ideal date at 24 months. Whenever within a data collection window the baby and family are contacted, the information collected about the baby's pulmonary health on that day may be used in the data collection window. If a baby and family is contacted more than once within a data collection window, the data closest to the ideal date are the data to be used. For example if a baby and family are contacted at 10 months and at 13 months, the data on the baby's pulmonary health collected at the 13-month visit are to be used for the 12-month visit. If data were not collected within a specified window but the baby and family are contacted in a subsequent window, the data collected that day should be processed for the current window, and inquiry made as to the baby's pulmonary health at the ideal time of the window in which data were not previously collected. If the baby's family can supply answers in response to the inquiry, those data should be entered for baby's pulmonary questionnaire CRF at the time of the ideal visit date for which information had been missing previously. For example, if the first follow-up contact for a baby is completed at 11 months adjusted age, the pulmonary health information collected about that day may be used for the 12-month visit, and answers to questions about the baby's pulmonary health at six months adjusted age may be used for late completion of the six-month visit information.*

6.3.6 Completion/Final Evaluation

- 22-26 months adjusted age
 - *Neurologic examination*: A developmental specialist or neurologist blinded to treatment assignment will complete a comprehensive neurologic examination ¹¹⁴ that includes a standardized assessment of reflexes, muscle tone and strength. The neurologic assessment will be classified as normal or abnormal and whether the child has cerebral palsy (CP) and CP severity as defined in the MOP.
 - *Gross Motor Function Classification Score (GMFCS)*: The degree of motor impairment will be assessed using the 5 level GMFCS ¹¹⁵.
 - *Bayley Scales of Infant and Toddler Development, 3rd edition (BSIDIII)*: A certified psychologist blinded to treatment assignment will complete the BSIDIII ¹¹⁶.
 - *Ages and Stages Questionnaire, Third Edition (ASQ3)*: Developmental milestones in five domains (communication, fine motor, gross motor, problem-solving ability, and personal-social functioning) will be assessed using a parent questionnaire administered by phone by study coordinators when a child is unable to return for in person assessments. This tool has high sensitivity and negative predictive value compared to the BSID-III at 24 months in preterm infants <32 weeks gestation ¹. The window for this assessment is from 19 months to 30 months with the ideal date at 24 months.
 - *Pulmonary questionnaire*: Data will be collected by structured parental interviews during the clinic visit. With permission, we will utilize the validated Tucson Children's Respiratory Study questionnaires that were designed to elicit a complete history of possible covariates such as family history of asthma or atopy, and important environmental (e.g., smoking) and infectious exposures and detailed interval respiratory health history. Recurrent wheezing and chronic cough will be defined as occurring more than twice per week.
 - *Review medical records*: Medical records will be reviewed and parents interviewed to assess vision and hearing. Records from subspecialty ophthalmology and audiology services will be reviewed to determine whether the child is receiving services for vision or hearing impairment and results of vision and audiological examinations.
 - *Neurodevelopmental impairment* will be assigned for any of the following: moderate to severe CP, bilateral blindness, bilateral hearing impairment requiring amplification, GMFCS ≥ 2 , BSIDIII cognitive or motor score <70, or ASQ $\leq 2SD$ on any domain.
- **Early Termination**: Subject will be classified as early termination if subject was terminated from study prior to 36 weeks PMA or discharge/transfer whichever comes first. A subject may be terminated for following reasons: patient enrolled, but not dosed; death; parent or LAR withdrawal of consent; investigator decision; or study chairman decision. Partial termination may occur when a parent requests no further study procedures, but can be followed for safety.

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

The subjects will be monitored for safety until discharge/transfer. The severity of adverse events and out of range laboratory values will be assigned as per the neonatal toxicity tables (Appendix D) and severity assessment of lab values (Appendix E).

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The safety and tolerability of oral azithromycin has been evaluated in 3995 patients aged 2 to 94⁹¹. The overall rate of side effects is 12% with gastrointestinal symptoms the most common. Ototoxicity, although rare, has been reported with IV azithromycin in adults and included reversible tinnitus and hearing loss⁹². A review of 43 open label, randomized pediatric trials performed outside the US that included 2655 patients treated with a 3 day course (10 mg/kg once daily) oral azithromycin for upper respiratory tract infections reported adverse effects in 8.7% subjects, primarily gastrointestinal symptoms⁹³. In a recent pharmacokinetics study of a single dose of IV azithromycin in 32 children ages 6 months to 16 years, there were no serious adverse events. Nausea was the most common treatment related symptom, reported in 25% patients. Although treatment with erythromycin in the first few months of life has been associated occasionally with the development of infantile hypertrophic pyloric stenosis (IHPS)⁹⁴, recent retrospective studies of a large cohort suggest that there is an increased risk of IHPS in infants >32 weeks gestation exposed to oral azithromycin or erythromycin^{95,96}. There was not an increases risk in preterm infants ≤32 weeks gestation.

The macrolides are associated with prolongation of the Q-T interval that can lead to serious ventricular arrhythmias. Recently, a retrospective study of a large Tennessee Medicaid cohort detected a small absolute increased risk of cardiovascular death (hazard ratio, 2.88; 95% CI, 1.79-4.63) in adults who took a five day course of azithromycin compared to individuals who took no antibiotics⁹⁸. There were 47 additional cardiovascular deaths per 1 million azithromycin courses. The increased risk for cardiovascular death was highest among patients with a high baseline risk for cardiovascular disease.

The implications of that study for azithromycin use in newborns and children are unclear. There is a single case report in the literature of cardiac arrest with suspected prolonged QT in a 9 month old who inadvertently received 50 mg/kg azithromycin intravenously over 20 minutes⁹⁹. Prolonged QT interval is rare among newborns with an incidence of the heritable long QT syndromes estimated between 1 per 3000 and 1 per 5000 births¹⁰⁰. Based on a retrospective study of prolonged QT interval in neonates, Villain and co-workers¹⁰¹ suggest that infants presenting with a QTc<0.5 second normalize their QTc over time while infants with a QTc>0.6 second are at risk for severe arrhythmias and sudden cardiovascular death. Prolonged QTc was not detected in any of the 171 preterm infants we screened for the 10 mg/kg and 20mg/kg single dose studies. However, we will continue careful screening of potential subjects for prolonged QT interval in the current protocol of multi-dose azithromycin. In consideration for safety, we will delay or discontinue study dosing if any subject has evidence of an arrhythmia on the cardiac monitor (they will all have continuous cardiopulmonary monitoring) or an ongoing proarrhythmic condition such as hypokalemia (serum potassium < 3.5 mEq/L), hyperkalemia (serum potassium >6.5 mEq/L on non-hemolyzed specimen), hypomagnesemia (total serum Magnesium ≤ 1.6 mg/dL), hypocalcemia (ionized

calcium < 3.0 mg/dL) or hypothermia (core temperature $\leq 35^{\circ}\text{C}$) until corrected by the clinical care team. We will also specifically record adverse events that may signal potential proarrhythmic effects in our study population such as sudden death, ventricular tachycardia, ventricular fibrillation and flutter, and seizures.

There are no known interactions of azithromycin with other drugs. The potential for these adverse outcomes will be carefully evaluated in the Phase IIb trial of azithromycin in preterm infants. Adverse events will be recorded on case report forms with specific focus on potential events with reported (expected) relationship to the drug (e.g. gastrointestinal symptoms, local IV site reactions, cardiac arrhythmias). Hearing loss will be assessed by a pre-discharge hearing screen. Clinical laboratory tests will be monitored throughout the first 28 d of the study. On study day 3 (± 1 d), 7 (± 2 d), 14 (± 2 d) and every 2 wks (± 2 d) until discharge or transfer, subjects will be assessed for morbidities associated with prematurity, and adverse events. Clinical laboratory testing (complete blood count with manual differential, electrolytes, liver function, and renal function) will be performed during the 24 hours pre- first dose, and study day 3 (± 1 d), and 7 (± 2 d), 14 (± 3 d), and 28 (± 3 d).

Cranial ultrasound and retinopathy of prematurity reports of all subjects will be reviewed for completeness and accuracy at the time of site visits or will be provided to the data coordinating center upon request. Independent review of CUS images will be requested if differences are noted in these outcomes by site or treatment assignment.

7.3 Adverse Events and Serious Adverse Events

- a. **Adverse event:** Any untoward medical occurrence in a patient or clinical investigation in which a subject is given a pharmaceutical product; does not necessarily have a causal relationship with such treatment, **or**
- b. Any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal (investigational) product; not necessarily related to the product.
- c. Change-from-baseline AEs can take 2 forms:
 - i. Appearance of a new symptom or sign, or
 - ii. Increased severity or frequency of an existing symptom or sign
- d. **Severity assessment:** Severity refers to the intensity of the event and is generally indicated as mild (Grade I), moderate (Grade II), severe (Grade III), or life-threatening (Grade IV). See appendices E and F, Neonatal Toxicity Tables for severity grading of common events and laboratory abnormalities. For events not listed, grading definitions are:
 - i. **Mild (Grade I):** mild transient symptoms, only requiring monitoring or symptomatic treatment, and clinically significant
 - ii. **Moderate (Grade II):** Moderate illness or condition which requires new or significantly altered specific therapy
 - iii. **Severe (Grade III):** Severe illness or condition: unresponsive to medical therapy.

- iv. **Life-threatening:** Life-threatening illness or condition. Complicated by acute, life-threatening metabolic or cardiovascular complications (such as circulatory failure, hemorrhage, sepsis); life-threatening physiological consequences; or need for intensive care or emergent invasive procedure (e.g. requires major surgery).
- e. **Causality** refers to the likelihood and extent that the investigational agent being studied contributed to the development of an AE. In making determination, consider temporal relationship to drug exposure, other possible exposures as etiology, known effects of the investigational agent, anticipated (*expected*) side effects derived from preclinical studies, and what is known about similar drugs (See drug insert).

Table 5. **Relatedness of AEs to an Intervention (Agent)**

Definite (must have all 4)

- Has a reasonable temporal relationship to the intervention
- Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions
- Follows a known pattern of response to intervention
- Disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure

Probable (must have 3)

- Has a reasonable temporal relationship to the intervention
- Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions
- Follows a known pattern of response to intervention
- Disappears or decreases with reduction in dose or cessation of intervention

Possible (must have 2)

- Has a reasonable temporal relationship to the intervention
- Could not have readily been produced by the subject's clinical state
- Could not readily have been due to environmental or other interventions
- Follows a known pattern of response to intervention

Unlikely (must have 2)

- Does not have a temporal relationship to the intervention
- Could readily have been produced by the subject's clinical state
- Could have been due to environmental or other interventions
- Does not follow a known pattern of response to intervention
- Does not reappear or worsen with reintroduction of intervention

- f. **Duration of event:** Onset of signs and symptoms as well as resolution of the event should be recorded, typically defined as the point when all signs and symptoms have subsided. Some events continue or change in severity over time. Capturing this information is also important. Changes in severity such as when an event improves from severe to mild should be recorded as separate events, with separate intensities and durations collected.
- g. **Adverse reaction:** Defined as an adverse event caused by a drug. Therefore, adverse reactions are a subset of all suspected adverse reactions for which there are reasons to conclude that the drug caused the event.
- h. **Suspected adverse reaction (21 CFR 312.32(a)):** Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.
- i. **Unexpected (21 CFR 312.32(a)):** An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure (or drug insert) or is not listed at the specificity or severity that has been observed; or, is not consistent with the risk information described in the general investigational plan or elsewhere in the protocol. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacologic properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (e.g. adverse events associated with other macrolides, but not specifically azithromycin such as pyloric stenosis). In addition, adverse events that would be anticipated to occur as part of the disease process are considered 'unexpected' for the purposes of reporting because they would not be listed in the investigator brochure.
- j. **Serious (21 CFR 312.32(a)):** An adverse event or suspected adverse reaction is considered 'serious' if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization, or prolongation of existing hospitalization, a persistent or significant incapacity, congenital anomaly/birth defect, or important medical event that may, based on appropriate medical judgment, jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the other outcomes listed in the definition.
- k. **Life-threatening (21 CFR 312.32(a)):** An AE or suspected adverse reaction is considered 'life-threatening' if, in the view of the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.
- l. **Morbidities Associated with Prematurity**

The population to be studied in this protocol is at high risk for morbidities associated with prematurity. Events listed below will, for the purposes of this clinical trial, be considered events

commonly associated with prematurity. The following morbidities associated with prematurity will be entered on the morbidities CRF.

- Air leak syndrome (e.g. pulmonary interstitial emphysema, pneumothorax, pneumomediastinum)
- Anemia of prematurity
- Apnea of prematurity
- Bradycardia
- Cystic periventricular leukomalacia (confirmed by cranial ultrasound)
- Feeding intolerance (e.g. gastric residuals, abdominal distension)
- Gastroesophageal reflux
- Gastrointestinal perforation (confirmed by radiology and surgical pathology to not be associated with necrotizing enterocolitis)
- Hyperbilirubinemia
- Necrotizing enterocolitis (confirmed by radiology)
- Patent ductus arteriosus (confirmed by echocardiography)
- Periventricular or intraventricular hemorrhage (confirmed by cranial ultrasound)
- Progressive hydrocephalus requiring shunting
- Respiratory Distress Syndrome
- Sepsis (culture-confirmed from sterile site e.g., blood, CSF, urine)
- Retinopathy of prematurity

7.4 Reporting Procedures

a. Reporting Procedures for all Adverse Events

- i. For adverse events that are level I or level II severity, a separate CRF AE form will be submitted for each occurrence. All AEs that occur after the initial study drug administration until discharge or transfer will be reported. All submitted AEs will be tabulated and submitted to DSMB, FDA annual report, and to each investigator for inclusion in annual reports to local IRBs.
- ii. A separate SAE form will be completed for each serious adverse event reported/observed until discharge or transfer. Each site investigator must report immediately (*within 5 business days*) to the sponsor (Viscardi) all serious adverse events, regardless of whether the investigator believes that they are drug related, including those events listed in the protocol as anticipated to occur in the preterm population independent of drug exposure (e.g. morbidities of prematurity), or in the drug insert as predicted to occur with the drug.

iii. Dr. Alex Agthe, study medical monitor will review event information and determine if event qualifies as SAE, severity, and relationship to drug. He may contact site research team for additional information to make this determination. He will be responsible for preparing FDA safety reports and notifying Dr. Viscardi, the study chairman and IND investigator-sponsor. Dr. Viscardi will be responsible for submitting the safety reports to the FDA within the time requirements, the UMB IRB, and to site investigators for submission to local IRBs. Dr. Viscardi will forward all initial and follow-up SAE reports to the DSMB chair Dr. Jonathan Davis with each occurrence. The DSMB chair may request treatment assignment from the Data Coordinating Center at the time of the initial report or will review treatment assignment in the monthly safety reports of cumulative SAEs. Reports of deaths will be submitted to the DSMB chair with each occurrence.

iv. FDA IND Safety Reports:

- a. Sponsor (Rose Viscardi, M.D.) must submit reports to FDA for those instances where there is evidence to suggest a causal relationship between the drug and the adverse event (i.e. adverse reaction of suspected adverse reaction).
- b. Reports may be submitted after
 1. a single occurrence because the event is uncommon, but is known to be strongly associated with drug exposure (e.g. anaphylaxis, Stevens-Johnson Syndrome)
 2. one or more occurrences: a single occurrence or a small number of occurrences of a SAE that is uncommon in the study population, but not commonly associated with drug exposure and event(s) occur in association with other factors strongly suggesting causation (e.g. strong temporal association).
 3. Aggregate analysis of specific events. Morbidities of prematurity are examples of protocol-specified serious adverse events. If the frequency of a specific SAE occurs in statistically greater frequency in the treatment than placebo arm and the events occur in association with other factors strongly suggesting causation (e.g. strong temporal association), a safety report will be submitted.
- c. Serious adverse event reports for any cases of NEC meeting criteria for seriousness (e.g., deaths, infants requiring intestinal resection) will be reported to the FDA regardless of relationship to study drug as an IND safety report no later than 15 days after the sponsor is informed of a NEC case that qualifies as serious. Fatal or life-threatening cases of NEC will be reported no later than 7 days after the sponsor is notified. With these NEC SAE reports, the investigational site where the event occurred and the number of trial participants enrolled in the trial will be identified. If available, the number of trial participants enrolled at the site where the SAE occurred will be provided.
- d. An expedited IND safety report will be submitted when any of the following criteria are met for **serious and unexpected suspected adverse reaction**:
 1. Suspected adverse reaction (reasonable possibility that the drug caused the adverse event)

2. Serious
 3. Unexpected
- e. Any unexpected and related fatal or life-threatening suspected adverse reactions must be reported to the FDA by telephone or FAX within seven (7) calendar days after the sponsor's initial receipt of the information. The FDA Medwatch 3500A mandatory form will be completed and submitted (www.fda.gov/medwatch).
 - f. A written report will be submitted to FDA within 15 calendar days for any serious unexpected adverse event considered related to study drug including those that have already been reported under the 7-day rule.

7.5 Follow-up for Adverse Events

Any relevant additional information that is submitted as follow-up to a specific SAE, will be submitted to FDA as a "Follow-up IND Safety Report" as soon as the information is available.

7.6 Safety Monitoring

An independent Data and Safety Monitoring Board (DSMB) has been appointed and reports to the PI. Members include a neonatologist, a biostatistician or a clinical trials epidemiologist, and infectious disease specialist. The DSMB will: 1) review and analyze the progress of the study, 2) approve amendments to the trial protocol, if warranted, 3) monitor the safety of the study treatments, 4) review data quality, 5) review interim analyses and recommend early stopping or continuation of the trial.

Monitoring for serious or unexpected adverse events (SAEs) will be performed throughout the hospitalization period or until 44 weeks PMA whichever comes first. The site investigator will submit completed SAE CRFs to Data Coordinating Center, the PI, and Dr. Alex Agthe, who will be the study medical monitor. Dr. Agthe will review event information and determine if event qualifies as SAE, severity, and relationship to drug. He may contact site research team for additional information to make this determination. He will be responsible for preparing FDA safety reports and notifying Dr. Viscardi, the IND investigator-sponsor. Dr. Viscardi will be responsible for submitting the safety reports to the FDA within the time requirements. The Data Coordinating Center will prepare summaries of these cases that will be submitted to the DSMB. SAEs that are study-related or are cause for urgent concern will be reported to the DSMB, the site IRBs, and FDA immediately after recognition of their importance. The DSMB may review patient safety based on an individual report or accumulating evidence. Cumulative reports of all AEs and SAEs will be provided at each DSMB meeting. The DSMB will meet before subject recruitment begins and will subsequently meet every 6 months in person or by teleconference to review the study progress and AE and SAE reports.

8. INTERVENTION DISCONTINUATION

Subjects may be withdrawn from the study for the following reasons: 1) at the request of the subject's parent(s) or guardian(s), or at the request of other legally authorized representative; 2) if, in the investigator's opinion, continuation in the study would be detrimental to the subject's well being; or 3) at the specific request of the Sponsor, the National Institute of Child Health and Human Development (NICHD). Although the decision to remove subjects from the study is the sole

responsibility of investigator, he/she is urged to discuss situations with Dr. Viscardi prior to removing participants from the study. If study drug is discontinued, subjects will continue to be followed for safety assessments. All randomized subjects will be included in the intent-to-treat analyses to the fullest extent possible. A 5% missing information rate (including early termination of study drug or consent withdrawal) is anticipated. In all cases, the reason for withdrawal will be recorded in the case report form (CRF) and in the subject's medical records. If the reason for withdrawal was an adverse event (AE), the subject will be followed up and the AE must be reported in accordance with the procedures in Section 7.4.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

To determine the microbiologic efficacy of IV azithromycin 20 mg/kg x 3 days to eradicate *Ureaplasma* from the preterm lung, we will conduct a multicenter, randomized, double-blind, placebo-controlled clinical trial. This endpoint is important to plan for a Phase III clinical trial to determine if ureaplasma eradication improves short-term (BPD) and long-term pulmonary outcomes.

We hypothesize that compared to placebo azithromycin 20 mg/kg x 3 days will improve survival without persistence of *Ureaplasma* in preterm infants with *Ureaplasma* respiratory colonization.

Primary Efficacy Variable: Survival without persistence of *Ureaplasma* respiratory tract colonization: To determine the efficacy of the IV azithromycin to eradicate *Ureaplasma* respiratory tract colonization, all randomized infants will have follow-up cultures of TAs if still intubated or NP aspirates if extubated at sampling times (2 and 5 days post third dose and 21 d of age). These samples will be frozen and shipped to UAB for analysis by culture and PCR. The MIC of azithromycin will be determined for each isolate. This will assess if *Ureaplasma* isolates develop azithromycin resistance over time or resistance varies among centers. Since *Ureaplasma* is only transmitted vertically, neonates who are positive by culture or PCR at any time point will be considered a colonized neonate. Although PCR may be more sensitive for detection of all *Ureaplasma* positive neonates, it does not distinguish between live and killed organisms. Therefore, we will define bacterial clearance as 3 negative cultures. The primary outcome will be survival with microbiological eradication of *Ureaplasma* defined as survival to discharge or transfer with 3 negative cultures obtained post-therapy.

Secondary Efficacy Variables:

Physiologic Test for BPD: Secondary outcomes will include the rate of physiologic defined BPD at 36 wks PMA in placebo controls (*Ureaplasma* colonized, and non-colonized) and azithromycin responders (*Ureaplasma* eradicated) vs non-responders (persistent *Ureaplasma*). For the BPD endpoint, the recently reported physiologic definition of BPD based on oxygen-saturation monitoring will be used¹¹¹.

Additional secondary endpoints will include overall mortality, incidence of co-morbidities of prematurity such as intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, bacterial and fungal nosocomial infection, pulmonary air leak, patent ductus arteriosus, retinopathy of prematurity, number of days of positive pressure ventilation, number of days of oxygen supplementation, use of postnatal steroids, and use of non-study antibiotics. The

treatment groups will be compared for the frequency of event outcomes and medians (treating deaths as bad outcomes) for durations.

9.2 Sample Size

Table 6. Power to detect a difference between survival with *Ureaplasma* eradication in treated and control groups using a two-sided .05 level test under various assumptions and true difference

Number of <i>Ureaplasma</i> -colonized subjects/group	Anticipated frequency of Survival without persistence of <i>Ureaplasma</i> among azithromycin group	Anticipated frequency of Survival without persistence of <i>Ureaplasma</i> among placebo group	Lower limit of power ¹	Upper Limit of Power ²
30	.70	.20	88%	93%
	.65	.20	83%	90%
	.60	.20	76%	83%
	.55	.20	67%	74%

¹ Power if there was perfect correlation between twins

² Power if there was no correlation between twins.

Sample Size Considerations: We chose our sample size (30 *Ureaplasma*-colonized subjects per group) in order to have good power to detect an effect of treatment on *Ureaplasma* respiratory tract eradication. To calculate the power we will have, we simulated thousands of data sets under various assumptions about the rates of survival without persistence of *Ureaplasma* in the control and intervention groups. For each simulated data set, we calculated a Mantel-Haenszel Chi Square, and determined the proportion of data sets for which the p-value was less than .05. These simulations were based on the assumption that 20% of the infants were in twin pairs. Since twins will be randomized to the same treatment group, and assuming the outcomes in twins are positively correlated, the power of the study regarding the treatment effect is less than what it would be had all the infants in the study been singletons (independent). To assess the range of power, the power was calculated for the two extremes of perfect correlation between twins and no correlation between twins. The actual correlation is likely to be between 0 and 1, so the actual power should be somewhere in between the limits as shown in Table 6. These power calculations also assume that 5% of the children will withdraw from the study before the endpoint is assessed. The actual power of our study will be somewhat higher than in these simulations because the actual analysis will glean information from both members of each twin pair using multiple outputation.

Power calculations were performed assuming an 80% survival rate, a 54% *Ureaplasma* colonization rate and a 25% rate of clearance among the placebo group to determine a sample size as shown in Table 6. Assuming that survival and *Ureaplasma* clearance are independent outcomes and 25% of the infected children will spontaneously clear the infection, then the expected rate of survival without microbiological persistence of *Ureaplasma* in the absence of antibiotic therapy is 0.2 (0.8 x 0.25). In Table 6, we provide the power we will have to detect differences in survival without microbiological persistence of *Ureaplasma* between azithromycin and placebo groups with a sample of 60 *Ureaplasma*-colonized infants under various assumptions,

using a two-sided, .05-level test. Thus we will have good power to detect effects that are typical of appropriately chosen antibiotics.

9.3 Definition of Populations

The primary analyses will be performed to test the hypothesis of no difference in survival and *Ureaplasma* eradication between subjects randomly assigned to placebo versus IV azithromycin. All primary analyses will be carried out according to the principle of intention-to treat. If the null hypothesis is not rejected, 95% confidence intervals will be constructed about the differences observed to demonstrate how large the differences could be in either direction.

9.4 Interim Analyses and Stopping Rules

Since the sample of *Ureaplasma* colonization is small (~30 in each arm), no interim analysis for efficacy is planned.

There will be no pre-specified stopping rules, but rather the DSMB will review SAEs as they occur. The DSMB chair may call a DSMB meeting to specifically review safety concerns. The DSMB recommendations may include study termination, study continuation with protocol modifications (e.g., change in eligibility criteria), or temporary suspension of enrollment until further evaluation is completed. The DSMB will review each death occurrence. Cumulative SAEs for each study arm will be provided to the DSMB to determine whether there are statistical as well as clinical concerns. These findings may be reviewed by the DSMB in a closed session and the findings will be used to determine what steps will be taken.

9.5 Outcomes

9.5.1 Primary Outcome

The primary outcome will be survival to discharge and eradication of *Ureaplasma* respiratory tract colonization.

9.5.2 Secondary Outcomes

Secondary outcomes will include the rate of physiologic defined BPD at 36 wks PMA in placebo controls (*Ureaplasma* colonized, and non-colonized) and azithromycin responders (*Ureaplasma* eradicated) vs. non-responders (persistent *Ureaplasma*). For the BPD endpoint, the recently reported physiologic definition of BPD based on oxygen-saturation monitoring will be used¹¹¹.

Additional secondary endpoints will include overall mortality, incidence of co-morbidities of prematurity such as intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, bacterial and fungal nosocomial infection, pulmonary air leak, patent ductus arteriosus, retinopathy of prematurity, number of days of positive pressure ventilation, number of days of oxygen supplementation, use of postnatal steroids, and use of non-study antibiotics. The treatment groups will be compared for the frequency of event outcomes and medians (treating deaths as bad outcomes) for durations.

9.6 Data Analyses

Analysis of Primary Outcome: The primary outcome will be survival to discharge and eradication of *Ureaplasma* respiratory tract colonization. The null hypothesis will be tested using the Mantel-

Haenszel chi-square test, taking into account different clinical sites. The test for differences in the primary outcome between the treatment groups will be conducted at an overall 2-tailed alpha-level of 0.05. Enrolled patients who have died will be counted as having adverse outcomes (i.e., as treatment failures) in the primary analysis. Death must be taken into account as an adverse outcome to avoid the possibility declaring an advantage for a treatment that is associated with improved *Ureaplasma* clearance but also with greater mortality. In addition to calculating a p-value, we will use a Mantel-Haenszel Odds Ratio to estimate the size of the effect of treatment and calculate its variance (and corresponding confidence interval) based on the method of Robins *et al.*¹¹⁷.

Although an issue not addressed in many neonatal clinical trials¹¹⁸, one complication to the analysis is the fact that some of the children will be in twin sets. This violates the assumption of independence required by the Mantel-Haenszel approach. To handle this complication, we will use the approach of “Multiple Outputation”¹¹⁹. This approach was previously used to address this complication in the NO CLD study²⁴. This approach involves the random selection of one child from each twin pair. Then the analysis is performed only using the randomly selected single child from each set of twins. These steps (random selection and subsequent analysis) are repeated 1000 times. The final estimate is the average of the estimates found in the multiple analyses. The variance of the multiple estimates can be used to appropriately adjust the variance of the final estimate to take into consideration the fact that each of the multiple analyses were only based on a subset of the data.

Prior to performing the primary outcome analysis, the Breslow-Day test for homogeneity of odds ratios will be used to assess if there is significant (at $\alpha = 0.05$) evidence of differences in the effect of treatment according to stratum or clinical site. The proposed approach to interactions will maintain the overall alpha level for the primary outcome analysis at 0.05. If a clinical site interaction that is not consistent with an assumption of homogeneity of treatment effect is detected, the clinical site's performance and results will be reviewed to determine the nature of the differences in treatment effects. Significant differences in stratum or clinical site treatment effects will be reported.

Secondary Analyses Including Subgroup Analyses: A similar approach (Mantel-Haenszel tests and estimation using multiple outputation) will be used to estimate the effect of the treatment on BPD rates. The BPD analysis will be done using all children (with and without *Ureaplasma*), and then separately in strata defined by the presence of *Ureaplasma*. In the unlikely event that there are important imbalances between the randomly assigned treatments in clinical or laboratory variables recorded (e.g., selected co-morbid states, concomitant medications), secondary analyses will be conducted using logistic regression to estimate treatment effects controlling for confounding factors. Model assumptions will be examined, and models will be tested for goodness of fit. If the assumptions required for a logistic regression model are rejected, alternate models will be explored. Again, the approach of multiple outputation will be used to obtain the final estimates and their variance.

Additional secondary analyses will assess the treatment effect on other outcome variables. Secondary outcomes to be considered include death which will be approached with survival analysis (time to event) methods; co-morbid outcomes which will be approached with logistic regression; number of days of treatment with positive pressure ventilation and number of days of oxygen supplementation which will be approached with rank or normal scores statistics to be able to assess outcomes with and without taking deaths into account.

Pulmonary and Neurodevelopmental Outcomes Analysis: Given the duration of follow-up required

for these aims, drop-out needs to be considered. So, initially, we will identify predictors of drop out, and compare the study groups with respect to drop-out rates. If there is moderate difference between the groups with respect to drop-out rates, then variables associated with drop-out will be controlled for in subsequent analyses.

The primary approach to analysis will be to compare the treatment groups with respect to the distribution of 6-month, *12 month*, and 22-26 month pulmonary outcomes (number and duration of hospitalizations, number of physician and emergency visits for respiratory illness, frequency of pulmonary medication use, and parental report of recurrent wheezing or chronic cough). Initially, these comparisons will be performed informally using side-by-side box plots and simple tables. More formal statistical analyses will be performed using the methods described for the primary outcome. Specifically, for binary outcomes (e.g., “ever hospitalized”), we will use Mantel-Haenszel methods, stratifying by center. If we need to control for confounding variables, we will analyze binary outcomes using logistic regression models including terms for center. For quantitative outcomes we will use multiple regression models including terms for center and potential confounders. To assess whether observed associations with treatment group are mediated by clearance of *Ureaplasma* infection, we will also fit models including a term for persistent infection. Final inferential statistics will be based on analyses using multiple outputation methods described above to account for correlation within twins. A similar approach for neurodevelopmental impairment at 22-26 months will be used to compare the difference for binary outcome survival free of neurodevelopmental impairment.

Pharmacokinetic Evaluation: The plasma concentration versus time data from the a) single 10 mg/kg (N=12), b) single 20 mg/kg (N=13) and c) multiple 20 mg/kg (N=30) will be compiled and analyzed using the nonlinear mixed-effects modeling software, NONMEM. The first-order conditional estimation procedure (FOCE) will be used for the analysis. Open one-, two-, and three-compartment structural disposition models with first order elimination, as implemented in the PREDPP library of models provided in the NONMEM software, will be evaluated to fit the data. Previously, a two-compartment structural model with the clearance and volume of peripheral compartment (V2) allometrically scaled on body weight (WT) best described the pharmacokinetics of i.v. azithromycin in preterm neonates. This model will be first used for the compiled PK data analysis. The selection of the final base model will be determined by the Akaike information criteria (AIC) and visual inspection of the goodness of fit scatter plots. The additive, proportional and exponential pharmaco-statistical models as well as a combination of the additive and proportional models will be evaluated to characterize the inter-individual variability and the residual random variability. During the covariate model-building process, the stepwise forward inclusion procedure will be applied to build the full model. The likelihood ratio test will be applied to discriminate the alternative nested models using the model selection criteria with a significance level of 0.05. Stepwise backward procedure will be applied to breakdown the full model to the final model with a stringent statistical criterion ($p < 0.01$). The improvement in the diagnostic scatter plots (population predicted versus observed concentrations, individual predicted versus observed concentrations, weighted residuals versus time, weighted residuals versus population predicted concentrations and ETA plots) as well as the decrease in the inter-individual and residual variances will be used to evaluate the goodness of fit throughout the model building process. Potential covariates include birth weight, gestational age, gender, race, current weight, length, body surface area, postnatal age and post-menstrual age (gestational age plus postnatal age). Consolidating data from 55 neonates [n=12 (single 10 mg/kg), n=13 (single 20 mg/kg) and n=30 (extending N for multiple 20

mg/kg)] will allow better examination of the influence of these covariates on the PK of azithromycin. Any potential drug/drug interactions caused by concomitant medications administered to mother in the 7 days prior to delivery (e.g., antenatal steroids, antibiotics, tocolytics) and to the subject from birth until 7 days (within the drug sampling period) post-azithromycin dosing (e.g., caffeine, diuretics, indomethacin, postnatal steroids) will also be examined. The final proposed PK model will be internally evaluated using posterior predictive check and simulating the data from which it was built. After the final model is developed, Bayesian estimates of the individual patients' PK parameters will be calculated, including plasma clearance, volume of distribution, maximum plasma concentration (C_{max}), time of C_{max} (T_{max}), area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$), the distribution rate constant, and elimination rate constant.

Pharmacodynamics evaluation: Microbiological outcome will be classified as eradication or persistence of *Ureaplasma* respiratory tract colonization. Eradication will be defined as all negative follow-up cultures and failure defined as the presence of one or more follow-up positive respiratory culture for *Ureaplasma*. Pharmacodynamic parameters of AUC_{24}/MIC_{90} , C_{max}/MIC , and %T>MIC will be determined for *Ureaplasma* culture positive subjects. Classification and regression tree (CART) analysis will be applied to determine breakpoints of PD indexes, e.g. AUC/MIC , and their statistical significances will be validated by Fisher's exact test. Logistic regression analysis will be performed to determine the probability of clinical success or bacterial eradication based on the significant predictors, such as AUC/MIC , and T%>MIC, etc.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data Capture: Data capture includes all procedures required to incorporate study data into a central database. Data capture and management of high quality data are key responsibilities of Dr. Tracy and the Data Management Core. The general principles that will be followed are: 1) continuous time windows to indicate ideal and permissible dates for follow-up visits; 2) permissible minimal and maximal time limits between specified data collection visits; 3) identical follow-up schedule for all treatment groups for measurement of variables used to assess outcome to avoid bias inherent in a schedule involving more frequent contact with one treatment group relative to the other; 4) tested data collection forms; 5) document contacts of participants, record reasons for missed visits via use of a missed visit form; 6) trained and certified data collectors; and 7) appropriate audit trails for all changes to data forms. The study coordinators at each site who are blinded to study treatment assignment, will be responsible for completing the case report forms. All data recorded by hand will be collected with paper case report forms designed and processed by a software application called Teleform (Cardiff, San Marcos, CA) that utilizes optical character recognition (OCR) and mark-sensitive technologies that allow data to be captured via electronic scanning rather than direct-keyed data entry. Teleform offers functions such as database validations, user-defined dictionary look ups, numeric range tests, data, currency and character-specific formatting, as well as "Always review" and "Entry required" field designations. Once data are processed through the Teleform suite of programs, they are committed directly to the secure project database.

Confidentiality

Subject confidentiality will be maintained in accordance with the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Only the subject number and letter code will be recorded in the CRF. Study findings stored on a computer will be stored in accordance with local data protection

laws. Patient data shall be stored with a unique numeric identifier to insure confidentiality. Names and addresses corresponding to each identification number will be kept in a secure file. Access to all computer files will be restricted to designated personnel through the use of passwords.

Direct Access to Source Data/Documents

The subjects' parents/guardians will be told that representatives of the NICHD, IRB, or FDA may inspect their child's medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

10.2 Data Management

Clinical site data collection

Clinical sites will be responsible for accuracy and completeness of CRFs and submission of completed forms to the Clinical and Translational Research Informatics Center within pre-specified windows. They will also be responsible for maintaining source documents and retention of original CRFs securely at their site. They will respond to edit checks and submit corrections on CRF modification/update/deletion form. Sample case report forms are provided in Appendix F.

Case Report Forms as Source Documents

Study personnel trained in the proper completion of case report forms (CRFs) will record study data on the CRFs and all forms will be signed and dated by the study personnel who completed the form. In some instances data for which there is no prior written or electronic record will be recorded on CRFs and these will be considered source documents. This will be limited to the following CRFs:

1. AZIP06 (Tracheal/NP Aspirate Samples Pre-dose)
2. AZIP07 (Physical Exam Study Day 1)
3. AZIP08 (Drug Administration) (with supplementary attachments)
4. AZIP09 (Vital Signs)
5. AZIP13 (Pharmacokinetics Plasma Sample Collection)
6. AZIP14 (Tracheal/NP Aspirate Samples Post Dose)
7. AZIP15 (Adverse Events) (with supplementary attachments)
8. AZIP16 (Serious Adverse Events) (with supplementary attachments)
9. AZIP17 (Physiologic BPD Assessment)
10. AZIP18 (Early Termination)
11. AZIP19 (Final Disposition Form)
12. AZIP 20 (Protocol Deviation Form)
13. AZIP21 (Pre-Discharge Questionnaire)
14. AZIP22 (Six Month Adjusted Age Questionnaire)
15. AZIP23 (CRF Modification/Update/Deletion Request)

Clinical and Translational Research Informatics Center

The CTRIC under the direction of Dr. Kate Tracy will provide data management services for the study. They will develop the case report forms, data edit and audits, and prepare monthly performance reports.

Data confidentiality, security, and backup: All data collection instruments are designed by the CTRIC to exclude the transmittal of personal identifiers to the study database. Participant names and other directly identifiable information will not be captured in the research database. Each participant will be assigned a participant ID (PID) and a randomly generated alpha code at the initiation of screening. These two identifiers will uniquely identify participants on all forms and only these identifiers will be used when communicating with clinical sites regarding data edits, data audits, and concerns regarding an individual participant. Study data will be stored in a suitably designed relational database housed on a password protected departmental server. Access to the database will be restricted to authorized personnel and require the user to enter an appropriately complex password that is changed on a regular basis. Any modifications, updates or deletions will be implemented only by the database manager, and only when the proper documentation has been provided by the PI. A record of each change to the database will be maintained in a transaction log to allow for recovery of the original data if needed. Incremental backups of the database are performed daily and the database in its entirety is backed up weekly and maintained on tapes that are moved to a fire-proof safe off location to guard against catastrophic loss. These tapes are kept for a minimum of 12 months. This backup mechanism allows for restoration of the server to any desired state in the past. Thus, in the event of a catastrophic mishap, the data system can be rolled back to a state just before the error occurred. All backups are stored in secure locations and on password-protected computers.

Quality assurance: As data are collected, validity and accuracy checks that will be pre-programmed will be performed. Post-capture validity checks may also be programmed to identify other data quality issues. Reports of missing forms will be produced, and percent of important variables missing will be counted. Queries will be generated for resolution at the data collection point level. There will be a timely flow of reported information so that the PI or her designate can take corrective action when needed to address data quality issues.

Data edits and audits: Edits are run on the database on a regular basis. Edits include checking for unusual or inconsistent values, as well as examining missing values. The PI or her designate is notified of the edits found and subsequently must make suitable updates to the forms, complete the necessary Modification/ Update/ Deletion (MUD) Log, and respond regarding resolution of the edits. Items previously queried in the data editing process that are legitimate (e.g., missing data from a previous visit that cannot be gathered at this point in time) are removed from future lists of data edits. Approximately 5% of the scanned forms are randomly selected and a list of requested forms is sent to the PI. Copies of the original forms are marked for re-scanning for comparison with the records in the existing database. The forms are also examined for other problems (e.g., improper documentation of changes made on the form). Discrepancies within the database must be resolved.

Performance monitoring: The performance of the clinical sites is monitored via a variety of reports that are produced on a regular basis to assist the PI and project staff in monitoring study progression and recruitment, including information on what forms have been completed for each participant at each assessment time point, summaries of data edits and audits, the number of patients screened over time, the number of patients enrolled over time, and the timeliness of submitting forms for processing. Reports will be stratified by clinical site and/or important covariates as appropriate.

10.3 Quality Assurance

10.3.1 Training

The principal investigator, Dr. Viscardi will train the study coordinators in the procedures for tracheal aspirate and nasopharyngeal culture collection and the room air challenge test. All study procedures will be reviewed during the investigator meeting to be held prior to initiating the study. The PI will conduct site initiation visits to all additional sites to review the manual of procedures and sample collection techniques.

For the neurodevelopmental assessments, a primary neurologic examiner will be identified who will certify annually and certify other examiners at their respective sites. Training DVDs of neurologic exams to include normal neurologic exam, CP, GMFCS \geq level 2, and neurodevelopmental impairment will be prepared and each primary examiner will score each exam independently and their inter-rater agreement with identified Gold Standard examiners will be determined.

10.3.2 Quality Control Committee

Drs. Viscardi and Terrin will review monthly performance reports for site-specific enrollment, CRF completion, data edits (number of error messages), sample collection, and protocol deviations. They will contact site research staff if specific problems are identified in order to improve study performance.

10.3.3 Metrics

The UAB Mycoplasma Diagnostic Laboratory will be the central laboratory for all *Ureaplasma* culture, PCR, and antibiotic susceptibility testing and will conduct all testing according to strict standard operating procedures. They will provide 10B broth to all sites for collection of respiratory specimens. Appropriate controls will be run with each culture and PCR to identify false positive and false negative results.

Assignment of physiologic BPD is an important secondary outcome. The investigators will be trained in the timed oxygen reduction test and all case report forms will be assessed for completion and accuracy. The PI will review whether the BPD assessment algorithm was applied accurately in each case of infant who survived until 36 weeks PMA.

10.3.4 Protocol Deviations

Protocol violations may jeopardize the study by breaching assurances made to the subjects or by diminishing the validity of the study. Major violations are those that endanger patients, such as failure to adhere to appropriate inclusion/exclusion criteria or undermine the conduct of the study such as failure to obtain blood and respiratory samples at the appropriate times. Minor violations are those that impede the progress of the study, such as not completing CRFs in a timely fashion. If blood draws or tracheal aspirate samples are outside the appropriate time range, an explanation will be documented. The principal investigator, site investigators and coordinators will problem-solve to limit future protocol violations. Protocol violations will be reported to the IRB and the DSMB.

10.3.5 Monitoring

The site PI will be responsible for assuring protocol compliance and data quality at their sites. Dr. Viscardi will visit each site annually to review records, consent forms and adherence to procedures as outlined in the Manual of Procedures.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document (Section 16.1) and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study.

11.2 Informed Consent Forms

If an infant qualifies, the PI or coordinator will obtain informed parental consent prior to initiation of study procedures. An EKG for QTc measurement will be obtained after consent if not done previously during the screening process. If the PI is also responsible for the clinical care of a potential subject, he/she will not participate in the consent discussion with the parents to avoid undue pressure on the parents to agree to the study. The information provided in the consent will cover the elements listed in the CFR Part 50.25 and be approved by the UMB Institutional Review Board (IRB). This includes the investigational nature and objectives of the trial; the procedures and treatments involved and their attendant risks, discomforts, and benefits; and the potential alternative therapies, alternative to not participate and right to withdraw without penalty will be explained. The study staff personnel will offer to answer any questions. Consent will be documented by parental signature on the IRB approved consent form. A copy will be given to each legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All Teleform entry and will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, and the NICHD.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCAM, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

Study Chair: The responsibilities of the Study Chair (Dr. Viscardi) are to: 1) Provide overall organization of the Clinical Consortium; 2) Serve as Chair, Steering Committee; 3) Oversee the subcontracted activities of the UAB Diagnostic Mycoplasma Laboratory, and the functional units within UMB (the UMB Coordinating Pharmacy, Data Management Core, UMB Pharmacokinetic Core, and UMB Cytokine Core Laboratory); 4) Work with the Clinical Sites, Laboratories and Cores to maximize collaboration and to arrange study meetings and to provide collaborators with information about the progress of the trial; 5) Hold the IND and submit study progress reports annually to the NICHD and FDA; and 6) Lead the presentation and publication of study results for the scientific and lay press.

Steering Committee: A Steering Committee will be responsible for overseeing the study. This committee will make major organizational and policy decisions. The committee will include the Study Chair, Deputy Chair (Dr. Terrin), Laboratory and Core Directors, and the Clinical Site Directors. This group will have quarterly conference calls and meet yearly prior to the annual Clinical Site Directors and Coordinators meeting.

Publications and Ancillary Studies Committee: A Publications and Ancillary Studies Committee will be formed from the study leadership (PIs and Laboratory/Core Directors) and leading Clinical Site Directors. This committee will review all proposals for and final versions of research abstracts, presentations, and manuscripts to be submitted to journals and national meetings. The committee will also review proposals for ancillary studies. Since the success of the trial depends upon the collaboration of the Clinical Sites, credit will be assigned to them (as the “AZIP Research Group”) in the authorship of reports from the study. Each Clinical Site Director and Clinical Site Coordinator will be named in an appendix to the main report.

13. PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by the policies and procedures developed by the Steering Committee.

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15. SUPPLEMENTS/APPENDICES

16. CONSENT FORM

Protocol Title: Azithromycin to Prevent Bronchopulmonary Dysplasia in *Ureaplasma*-Infected Preterms: A Phase IIb Randomized, Placebo-Controlled Trial of Azithromycin to Eradicate *Ureaplasma* Respiratory Tract Infection in Preterm Infants

Study No.: HP-00054998

Principal Investigator: Rose M. Viscardi, M.D.
Contact Information (410-328-6003)

Sponsor: National Institutes of Health

You are being asked to allow your baby to participate in this research study because your baby was born early (prematurely). Your baby's participation in this study is voluntary and you may ask questions at any time. If you are consenting for someone else- a child or someone unable to provide consent themselves- then the word "you" means that person.

PURPOSE OF STUDY

Approximately 54% of babies born very early (before the 27th week of pregnancy) have a lung infection with the bacteria *Ureaplasma urealyticum*. This infection may lead to a chronic lung condition called Bronchopulmonary Dysplasia (BPD). There is no known effective treatment for this infection in preterm babies. Azithromycin is an antibiotic approved for use by the Food and Drug Administration (FDA) to treat infections caused by other bacteria in older children and adults, but has not been approved by the FDA for treating *Ureaplasma* infections in premature babies. This research study is being done as part of the FDA approval process for the use of azithromycin for this purpose. Azithromycin has been shown to be effective against *Ureaplasma* in test tube tests. By measuring blood levels of the drug in 40 preterm babies who received azithromycin as a single dose of either 10 mg or 20 mg or 3 doses of 20 mg for about every 2 pounds of body weight as part of a research study, we found that the 3 doses of 20 mg for

about every 2 pounds of body weight was most effective to eliminate the *Ureaplasma* bacteria from the lungs of babies who had the infection. There were no side effects attributed to the antibiotic.

Babies with and without the *Ureaplasma* lung infection may participate in this study to evaluate azithromycin in preterm babies.

The purpose of this study is to evaluate whether 3 doses of azithromycin 20 mg/kg daily based on body weight will effectively eliminate the *Ureaplasma* bacteria from the lungs of babies who have the infection and to make sure azithromycin is safe in very preterm babies. To determine the effectiveness of azithromycin, this study will compare the active drug to a placebo. A placebo is an inactive substance made to look like an active medicine. Your child will either get the study drug, azithromycin or a placebo. Researchers use a placebo to see if the study drug works better or is safer than not taking anything. In addition, the study will find out how 3 doses of azithromycin or placebo affect markers (indicators) of inflammation in the lung in all treated babies. Finally, the study will find out how 3 doses of azithromycin or placebo affect lung health status at 6 months adjusted age (6 months plus number of weeks born early), 12 months adjusted age (12 months plus number of weeks born early) by a phone interview and lung health and development status at 22-26 months adjusted age (22-26 months plus number of weeks born early) during a clinic visit.

A total of 140 subjects at 7 medical centers will be asked to participate in this study. Your baby will be one of approximately 40 subjects to be asked to participate at this location.

The research will be conducted at the following location(s): University of Maryland Baltimore, University of Maryland Medical System, and other sites.

PROCEDURES

This is a double-blinded study, which means that neither you or the study doctor or the study staff will know which treatment you are receiving. However, in an emergency, study doctor can get this information.

If you agree to allow your baby to participate in this study and your baby is eligible for participation you will be asked to read and sign this document. If you agree for your baby to participate, the following procedures will be performed.

1) Nose or respirator tube sample: The fluid from the baby's nose or respirator tube will be tested for the *Ureaplasma* bacteria 5 times: twice before the baby gets the study drug, once two days after the baby gets the study drug, once from 4 to 5 days after the drug, and once when the baby is 21 days old, or right before the baby is discharged, whichever comes first. These samples will be sent to the Diagnostic Mycoplasma Laboratory at the University of Alabama, Birmingham for bacterial testing. Fluid samples collected at the same times will be tested for indicators of inflammation. These samples will be obtained during routine suctioning (cleaning) of your baby's nose or the tube in your baby's windpipe that is connected to the breathing machine. You will not be contacted with the results of the bacteria tests or indicators of inflammation testing.

2) Electrocardiogram (EKG): If the baby's heartbeat is not regular on his/her bedside monitor, an electrocardiogram test (test that measures the electrical activity of the heartbeat) will be done before study drug is given. If a special pattern called prolonged QT interval is seen on the EKG, the drug will not be given. If the baby's blood potassium, magnesium, or calcium levels are low or the blood potassium level is high, the drug will not be given until the blood levels are corrected.

3) The treatment your child receives will be chosen by chance, like flipping a coin. Neither you nor the study doctor will choose what treatment your child gets. Your child will have an equal chance of being given each treatment. Neither you nor the study doctor will know which treatment you are getting. Your baby will receive either the study drug (azithromycin) or placebo (an inactive substance made to look like an active medicine) once a day for 3 days by infusion, which means that it will be given through an intravenous line (line inserted in a vein). Your baby will receive the study drug dose of 20 mg/kg based on their body weight or the same volume of placebo. Each dose will be given over a 1 hour period of time.

4) Test for BPD: If your baby is still in the hospital between 35 to 37 weeks gestation (1 month before the due date) and is still receiving extra oxygen through a tube to the nose, he/she will be tested to find out if he/she is ready to stop

the extra oxygen. The extra oxygen will be decreased every 5 minutes by 2% at a time until the extra oxygen is stopped and the baby is breathing room air for 30 minutes. The decrease in oxygen will be stopped if the baby's oxygen levels are 80-89% continuously for 5 minutes or less than 80% for 15 seconds at any time. Your baby will be monitored continuously throughout the test for BPD by study staff and returned to their starting oxygen treatment as soon as the test is done.

5) Medical Records: The study doctors will review your baby's medical record. The study doctors will review the mother's medical record to make note of the treatments she received within one week of delivery.

6) Complete questionnaires: Before your baby leaves the hospital, a study staff member will contact you to ask questions concerning family history of lung problems such as asthma, and exposures such as smoking in the home. You will be asked to provide the name and contact information for your child's health care provider and permission to contact them after your child reaches 6 months adjusted age. Adjusted age is the age your baby would be if born on his or her due date. At 6 months adjusted age, 12 months adjusted age, and between 22 and 26 months adjusted age, a study member will contact you by phone to obtain a lung health history for your child by asking about breathing problems, illnesses, hospital stays, emergency room or doctor visits, and prescribed treatments. We will call your health care provider at this time to confirm your child's medical history.

7) Development assessment: When your baby is between 22 and 26 months adjusted age, the study staff will schedule a visit at the Mount Washington Pediatric Hospital Outpatient Clinic located at 1708 W. Rogers Ave., Baltimore, MD to evaluate your child's development. He/she will have a complete physical exam including a neurologic exam of his/her muscle tone, strength, and reflexes. The developmental test will evaluate your child's developmental milestones. You will receive a report about your child at the end of this visit.

Your baby will receive the same medical care whether s/he joins the study or not. In addition to the regular care for prematurity, the study doctors and nurses will follow your baby closely to make sure the infusions are safe.

If the *Ureaplasma* bacteria is obtained from the baby's nose or respirator tube culture, your child may be eligible for future studies of *Ureaplasma* bacteria as a result of his/her participation in this study. However, you may decline permission for future use of his/her samples and still agree to their participation in this study.

I _____ agree _____ do not agree to have my child's bacteria samples stored for possible use in future studies (please initial).

Please _____ do _____ do not contact me for future studies (please initial).

WHAT ARE MY RESPONSIBILITIES IF I TAKE PART IN THIS RESEARCH?

If you take part in this research, you will be responsible to:

- Ask questions about anything you do not understand
- For your child to complete all research procedures unless you formally request to withdraw from the study
- Bring your child to the follow-up clinic for scheduled visit between 22 and 26 months adjusted age
- Notify study staff of all changes to your contact information including your address, phone numbers, and e-mail address

POTENTIAL RISKS/DISCOMFORTS:

Fluid from the baby's nose or respirator tube will be collected at the time of routine suctioning by the baby's nurse. The discomfort from this procedure is minimal.

As the extra oxygen is decreased for the BPD test, the baby may experience brief periods of lower oxygen levels or brief periods of lower heart rate. The baby's oxygen level will be monitored for the entire BPD test and the extra oxygen will be adjusted if needed.

The most common side effects reported in older children and adults treated with azithromycin are gastrointestinal symptoms such as nausea, vomiting, and diarrhea (about 10%), pain at the injection site (6%), and less often vaginitis (vaginal discharge or infection), abdominal (belly) pain, loss of appetite, rash, and itching. Fewer than 1 in 100 babies had side effects of burping, passing gas, stomach upset, mouth yeast infection (thrush), headache, sleepiness, bronchospasm (temporary narrowing of airways into the lungs), and funny taste. In most cases these symptoms were mild. Rarely, heartbeat abnormalities, allergic reactions, pseudomembranous colitis (inflammatory condition of the large intestine due to imbalance of "good" and "bad" bacteria in the intestine) and reversible hearing loss have been reported. Blood tests for liver and kidney function were out of normal range for 6% or less in older children and adults treated with azithromycin. Low numbers of white blood cells (cells that fight infection) and platelets (cells that form blood clot) have occurred in less than 1% of treated individuals. Laboratory blood tests went back to normal in other clinical trials of azithromycin. Side effects seen in infants with oral (by mouth) azithromycin and similar antibiotics include pyloric stenosis (narrowing of the stomach connection to the small intestine). Pyloric stenosis can lead to stomach blockage requiring surgery to relieve the blockage. Since azithromycin is an antibiotic, there is the risk of other bacteria that are resistant to the antibiotic starting a new illness.

There is a risk to confidentiality. This risk will be minimized by storing your baby's personal and medical information collected as part of this study in a secure location (locked file in the PI's office) and electronic data will be password protected.

There are no other additional known risks and discomforts associated with this study other than those of the infusion over and above the risks of being extremely premature and in the NICU. There may be unforeseen risks of participation in the study. If you have any questions about the risks, please ask your baby's doctor, the study doctor, or the study staff.

POTENTIAL BENEFITS

Your child may or may not benefit by taking part in this study. There is no guarantee that your child will receive direct benefit from his/her participation in this study. The benefits of participating in this study may be a chance that azithromycin may decrease inflammation in the lungs or help get rid of the *Ureaplasma* bacteria from your baby's lungs if there is an infection. Your baby's participation may provide information that may benefit other babies with *Ureaplasma* lung infection in the future by helping to determine whether 3 doses of azithromycin is effective in eliminating the infection. Still, your baby may get no direct benefit from this study. You need to decide if your child's participation in this research study is in your child's best interest.

ALTERNATIVES TO PARTICIPATION

The following alternative procedures or treatments are available if you choose not to allow your baby to participate in this study: There is no approved drug to treat *Ureaplasma* lung infections in babies now.

COSTS TO PARTICIPANTS

It will not cost you anything to take part in this study.

PAYMENT TO PARTICIPANTS

You will receive a \$50 gift card after the completion of your child's neurodevelopmental assessment and lung health questionnaires at the end of the study when your child is 22-26 months adjusted age.

CONFIDENTIALITY AND ACCESS TO RECORDS

All of the baby's samples will be coded with a study number rather than their name or other identifier. We will keep your name and contact information on file to contact you at 6 months adjusted age, 12 months adjusted age and between 22 to 26 months adjusted age and in case we need to notify you of any important information about the study. Only the principal investigator, Dr Rose Viscardi and her staff will have access to your baby's private information. The data from the study may be published. However, you will not be identified by name.

Efforts will be made to limit your personal information, including research study and medical records, to people who have a need to review this information. People designated from the institutions where the study is being conducted and people from the sponsor will be allowed to inspect sections of your medical and research records related to the study. Everyone using study information will work to keep your personal information confidential. Your personal information will not be given out unless required by law. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization, the Food and Drug Administration, Department of Health and Human Services, and National Institutes of Health. The monitors, auditors, the IRB, and the Food and Drug Administration will be granted direct access to your medical records for verification of the research procedures and date. By signing this document you are authorizing this access.

A description of this clinical trial is available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

RIGHT TO WITHDRAW

Your participation in this study is voluntary. You do not have to take part in this research. You are free to withdraw your consent at anytime. You will be told of any significant new findings which develop during the study which may affect your willingness to participate in the study. There are no adverse consequences (physical, social, economic, legal, or psychological) if you decide to withdraw from the research. Refusal to take part or to stop taking part in the study will involve no penalty or loss of benefits to which you are otherwise entitled. If you are an employee or student, your employment status or academic standing at UMB will not be affected by your participation or non-participation in this study. If you decide to stop taking part, or if you have questions, concerns, or complaints, or if you need to report a medical injury related to the research, please contact the investigator Dr. Rose Viscardi at 410-706-1913 during the day and at 410-328-6003 after hours. If you withdraw from this study, already collected data may not be removed from the study database. You will be asked whether the investigator can collect data from your routine medical care. If you agree, this data will be handled the same as research data.

CAN I BE REMOVED FROM THE RESEARCH?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include a treatment becomes available for him/her that may be better for him/her than the care available in the study, or he/she has a serious reaction during the study. The Food and Drug Administration or the sponsor can also end the research study early. The study doctor will tell you about this and you will have the chance to ask questions if this were to happen.

UNIVERSITY STATEMENT CONCERNING RESEARCH RISKS

The University of Maryland, Baltimore (UMB) is committed to providing participants in its research the rights due them under State and federal law. You give up none of your legal rights by signing this consent form or by

participating in the research project. This research has been reviewed and approved by the Institutional Review Board (IRB). Please call the Institutional Review Board (IRB) if you have questions about your rights as a research subject.

Participating in research may result in an injury, as explained above. If you suffer an injury directly related to your participation in this project, UMB and/or one of its affiliated institutions or health care groups will help you obtain medical treatment for the specific injury and provide referrals to other health care facilities, as appropriate. UMB and/or its affiliated institutions or health care groups will not provide you with financial compensation or reimbursement for the cost of care provided to treat a research-related injury or for other expenses arising from a research-related injury. The institution or group providing medical treatment will charge your insurance carrier, you, or any other party responsible for your treatment costs. If you incur uninsured medical costs, they are your responsibility. The HRPO will assist you in contacting the sponsor if you have an injury caused by the sponsor's drug or device under study. Uninsured medical costs to treat research related injuries not caused by the drug or device under study are your responsibility. The study staff can give you more information about this if you have a study injury.

By signing this Consent Form, you are not giving up any legal rights. If this research project is conducted in a negligent manner and you are injured as a direct result, you may be able to recover the costs of care and other damages from the individuals or organizations responsible for your injury.

If you have questions, concerns, complaints, or believe you have been harmed through participation in this research study as a result of researcher negligence, you can contact members of the IRB or the Human Research Protections Office (HRPO) to ask questions, discuss problems or concerns, obtain information, or offer input about your rights as a research participant. The contact information for the IRB and the HRPO is:

University of Maryland School of Medicine
Human Research Protections Office
620 W. Lexington Street, Second Floor
Baltimore, MD 21201
410-706-5037

Signing this consent form indicates that you have read this consent form (or have had it read to you), that your questions have been answered to your satisfaction, and that you voluntarily agree to participate in this research study. You will receive a copy of this signed consent form.

If you agree to participate in this study, please sign your name below.

<hr style="border: none; border-top: 1px solid black;"/> <div>Participant's Signature</div> <div>Date: _____</div>	<hr style="border: none; border-top: 1px solid black;"/> <div>Signature of Parent/Guardian <i>(When applicable)</i></div> <div>Relationship: _____</div> <div>Date: _____</div>
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Investigator or Designee Obtaining Consent
Signature

Date: _____

Time: _____

Witness*

Date: _____

16.1 RESEARCH CONSENT FORM

17. OTHER APPENDICES

- A. Screening Log
- B. Study Schedule
- C. Effective FiO₂ Table
- D. Neonatal Toxicity Table
- E. Severity Assessment of Clinical Laboratory Values
- F. Case Report Forms
- G. Ages and Stages Questionnaires 22 month-27 months